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ABSTRACT

This manual is intended primarily to train seed collectors, seed-plant managers, seed analysts, and nursery managers, but can serve as a resource for any training course in forest regeneration. It includes both temperate and tropical tree species of all intended uses and covers the following topics: seed biology, seed collection, seed handling, seed-quality evaluation, seed protection, seed basics for nurseries, seed programs, and practical exercises. Contains 91 references. (Author/JRH)

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Tree Seed Technology Training Course

Student Outline

F. T. Bonner, J. A. Vozzo, W. W. Elam, and S. B. Land, Jr.

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SUMMARY

This manual is intended primarily to train seed collectors, seed-plant managers, seed analysts, and nursery managers, but it can serve as a resource for any training course in forest regeneration. It includes both temperate and tropical tree species of all intended uses. The manual covers the following topics: seed biology, seed collection, seed handling, seed-quality evaluation, seed protection, seed basics for nurseries, and seed programs. It also includes practical exercises.

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Tree Seed Technology Training Course Student Outline

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Introduction

GOALS OF THE COURSE

The objective of this course is to provide a basic understanding of the following topics:

A. Reproductive cycles, from flowering through seed germination
B. Seed origin
C. Seed collection
D. Seed maturity
E. Collection and postharvest care
F. Seed extracting, cleaning, and conditioning
G. Insect and disease problems
H. Seed storage
I. Seedlot sampling
J. Tests for moisture, purity, weight, germination, and vigor
K. Rapid viability estimates
L. Seed test results
M. Seed handling in nurseries
N. Seed programs
O. Seed labeling and certification
P. Germplasm conservation
Q. Seed center design and staffing
R. Applied seed research

SCOPE

The emphasis in this course will be on indigenous, multipurpose tree species suited for forestry and rural agroforestry. However, exotics are included, because fast-growing exotics have a definite place in forestry programs.

SOURCES OF INFORMATION AND TECHNICAL HELP

Sources of information and technical help include journals, reference books, international organizations, regional organizations, and research institutions.

Journals

Seed Science and Technology
International Seed Testing Association (ISTA)
Reckenholz, P.O. Box 412, CH-8046
Zurich
Switzerland

Journal of Seed Technology
Association of Official Seed Analysts (AOSA)

Seed Abstracts
CAB International Information Services
Wallington, Oxon OX 10 8 DE
UK

Agroforestry Abstracts
(same as above)

New Forests
Dr. Mary Duryea, Editor-in-Chief
Department of Forestry
118 N-Z Hall
University of Florida
Gainesville, FL 32611
U.S.A.

Indian Forester
(includes seed technology articles)

Indian Journal of Forestry
(includes seed technology articles)

Journal of Tropical Forest Science
Business Manager
Forest Research Institute of Malaysia
P.O. Box 201
Kepong, 52109 Kuala Lumpur
Malaysia

Commonwealth Forestry Review
Commonwealth Forestry Institute
11 Keble Road
Oxford
UK

Pakistan Journal of Forestry
Editor
P.O. Pakistan Forestry Institute
Peshawar, N.W.F.P.
Pakistan

Canadian Journal of Forestry Research
Editor
Forestry Canada
P.O. Box 490
Sault Ste. Marie, Ontario
P6A 5M7 Canada

Forest Science
Society of American Foresters
5400 Grosevenor Lane
Bethesda, MD 20814-2198
U.S.A.

Reference Books

The following reference books provide information and technical assistance:

Bewley and Black 1982
Chin and Roberts 1980
Murray 1984a, b
Schopmeyer 1974
von Carlowitz 1986
Willan 1985

International Organizations

Food and Agriculture Organization (FAO) of the United Nations
Forest Resources Development Branch
Forest Resources Division
Via delle Terme di Caracalla
I-00100 Rome
Italy

International Union of Forestry Research Organizations (IUFRO)
Secretariat
Schonbrunn
A-1131 Vienna
Austria

IUFRO Seed Problems Project Group
Current Chair: Dr. D.G. Edwards
Forestry Canada
Pacific Forestry Centre
506 West Burnside Road
Victoria, BC
V8Z 1M5 Canada

International Seed Testing Association (ISTA)
ISTA Secretariat
Reckenholz, P.O. Box 412
CH-8046 Zurich
Switzerland

Regional Organizations

F/FRED Coordinating Unit
c/o Kasetsart University
Faculty of Forestry
P.O. Box 1038, Kasetsart Post Office
Bangkok 10903
Thailand

Organization for Economic Cooperation and Development (OECD)
Directorate for Agriculture and Food
Paris
France

Research Institutes

ASEAN-Canada Forest Tree Seed Centre
Mauk Lek, Saraburi
Thailand

Nitrogen Fixing Tree Association
P.O. Box 680
Waimanalo, HI 96734
U.S.A.

International Council for Research in Agroforestry (ICRAF)
P.O. Box 30677
Nairobi
Kenya

DANIDA Forest Seed Centre
Krogerupvej 3A
DK 3050, Humlebaek
Denmark

Forest Research Centre
P.O. Box HG 595
Highlands, Harare
Zimbabwe

Centre National de Semences Forestieres
PB 2682, Ouagadougo
Burkina Faso

CSIRO Division of Forest Research
P.O. Box 4008
Queen Victoria Terrace
ACT 2600, Canberra
Australia

Commonwealth Forestry Institute (CFI)
University of Oxford
Department of Forestry
Oxford
UK

USDA Forest Service
Tree Seed Research Unit
Forestry Sciences Laboratory
P.O. Box 906
Starkville, MS 39759
U.S.A.

USDA Forest Service
National Tree Seed Laboratory
Rt. 1 Box 182-B
Dry branch, GA 31020
U.S.A.

USDA Forest Service
Institute of Tropical Forestry
University of Puerto Rico, Agricultural Experiment Station
P.O. Box 25000
Rio Piedras, PR 00928-2500
U.S.A.

Centro Agronomico Tropical de Investigacion y Ensenanza (CATIE)
Turrialba
Costa Rica

Petawawa National Forestry Institute
Box 2000
Chalk River, Ontario
K0J 1J0 Canada

Biology

I. Flowering, Pollination, and Seed Maturation

A. Introduction

Knowledge of the seed biology of a tree species is essential to successful seed production and handling. The sexual life cycle must be known to plan for genetic improvement, production, collection, conditioning, storage, and planting of the seeds.

B. Objectives

1. Define common terms used to describe life cycles of plants.
2. Describe the general sexual cycle, flower structure, seed structure, and origin of the fruit of gymnosperms.
3. Describe the general sexual cycle, flower structure, seed structure, and origin of the fruit of angiosperms.
4. Identify primary differences between angiosperm and gymnosperm sexual cycles.
5. Describe the general development of fruits and seeds.

C. Key Points

The following points are essential for understanding flowering, pollination, and seed maturation:

1. A plant's life cycle is the time required to grow from zygote to seed production; there are two developmental cycles – a sexual cycle and an asexual cycle.
2. Knowledge of the sexual cycle is required for:
   a. tree-breeding programs
   b. seed orchard management
   c. seed collection
   d. seed conditioning and storage
   e. nursery management
3. The gymnosperm life cycle follows this order:
   a. naked seed
   b. seedling
   c. mature sporophyte
   d. strobili (cones)
   e. microspore and megaspore mother cells
   f. meiosis
   g. microspores and megaspores
   h. male and female gametophytes
   i. pollination
   j. single fertilization
   k. zygote and gametophytic tissue
   l. embryo
   m. naked seed on ovulate cone scale
4. The angiosperm life cycle differs from the gymnosperm life cycle in having:
   a. seeds enclosed in fruit (ripened ovary)
   b. true flowers rather than strobili
   c. double fertilization
   d. triploid endosperm tissue rather than haploid female gametophytic tissue in the seed

D. Definition of Terms

1. **Life cycle** – the time required to progress from zygote to seed production.
2. **Genotype** – the genetic makeup of a cell nucleus or an individual.
3. **Phenotype** – the external appearance of an organism.
4. **Mitosis** – nuclear (and usually cellular) cell division in which the chromosomes duplicate and divide to produce two nuclei that are identical to the original nucleus.
5. **Meiosis** – two successive nuclear divisions in which the chromosome number is halved and genetic segregation occurs.
6. **Pollination** – transfer of pollen grains from the anther or microsporophyll to the stigma or ovule.
7. **Fertilization** – fusion of sperm and egg (and also sperm with two polar nuclei to form endosperm in angiosperms).
8. **Diploid** (2N) – two sets of chromosomes in a cell nucleus.
9. **Haploid** (1N) – one set of chromosomes in a cell nucleus.
10. **Fruit** – a ripened ovary, sometimes including accessory flower parts, that surrounds the seed in angiosperms.
11. **Seed** – a ripened ovule that consists of an embryo, its stored food supply, and protective coverings.
12. **Mature seed** – a seed that can be removed from the tree without impairing the seed's germination.

E. Life Cycles

An understanding of life cycles is needed because:

1. Sexual and asexual systems reproduce genetically different populations.
2. Knowledge of the asexual cycle is needed before vegetative propagation can be used.
3. Knowledge of the sexual cycle is needed for successful tree breeding and seed production.

F. Angiosperm and Gymnosperm Sexual Cycles

1. **All tree species** are seed-producing plants (division, Spermatophyta) and belong to either the class Gymnospermae or Angiospermae.
   a. Angiosperm seeds are enclosed in carpels.
   b. Gymnosperm seeds are borne naked on scales.

c. Seeds of nonconiferous gymnosperms are borne singly.

2. **Gymnosperm life cycle** (fig. 1)
   a. Sporophyte
   b. Strobilus or cone, including:
      (1) reproductive short shoot
      (2) staminate cone (male)
      (3) ovulate cone (female)
      (4) gymnosperms may be either monoecious (female and male strobili on same tree) or dioecious (tree has only one sex).
   c. Meiosis and gametophytes
   d. Fertilization
   e. Seed (fig. 2)
      (1) Develops from the fertilized ovule.
      (2) Contains an embryo (cotyledons, hypocotyl, radicle), a seedcoat, storage tissue, and sometimes a seed wing.
   f. Fruit
      (1) Gymnosperms do not have true "fruits."
      (2) Gymnosperm seeds are enclosed by the following structures:
         (a) dry ovulate cones (e.g., *Abies*,

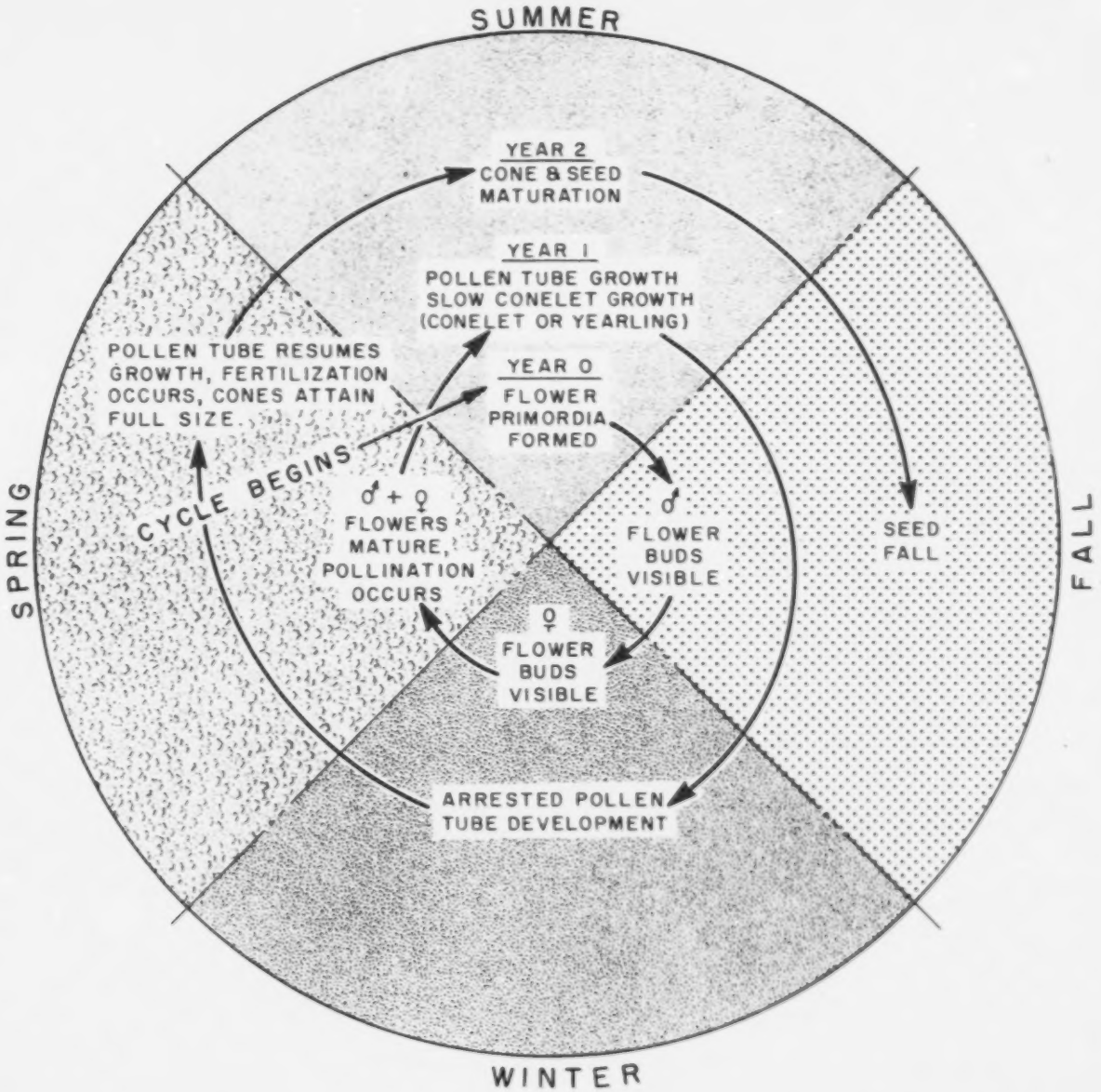

Figure 1.—*Life cycle of a gymnosperm* (Pinus *spp.*) *(Bonner 1991b).*

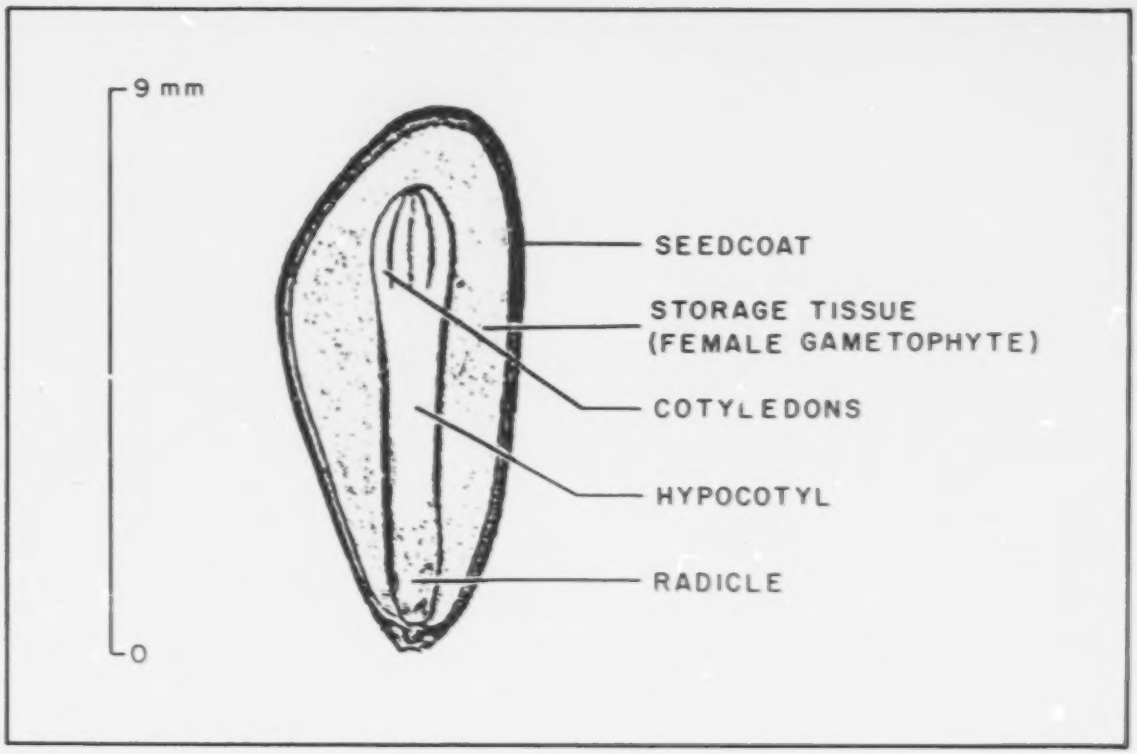

Figure 2.—*Cross section of a typical mature gymnosperm seed* (Pinus ponderosa) *(adapted from Krugman and Jenkinson 1974).*

Araucaria, Cupressus, Pinus, and *Tsuga*)
(b) fleshy, arillike structures (e.g., *Ginkgo, Taxus,* and *Torreya*)
(c) berrylike ovulate cones (e.g., *Juniperus*)

3. **Angiosperm life cycle**
   a. Sporophyte
   b. Flower—a short shoot with sterile and reproductive leaves.
      (1) Sterile leaves include:
         (a) sepals
         (b) petals
         (c) perianth
      (2) Reproductive leaves include:
         (a) stamen (male)
         (b) carpel (female)
         (c) pistil, a collective term that describes visible female structures
      (3) Receptacle
      (4) There are perfect flowers, imperfect flowers, and polygamous flowers
   c. Meiosis and gametophytes
   d. Fertilization
   e. Seeds
      (1) Develop from the double-fertilized ovules
      (2) Contain an embryo (cotyledons, hypocotyl, radicle), storage tissue, seed coat, and sometimes other seed coverings
      (3) May be endospermic or nonendospermic
   f. Fruits
      (1) Develop from the matured ovary
      (2) Enclose the seed (matured ovule)
      (3) Are difficult to separate from the seeds

4. **Sexual cycles**—The gymnosperm and angiosperm sexual cycles differ in four ways:
   a. In gymnosperms, seeds are not enclosed in the ovary, and flowers are unisexual; in angiosperms, seeds are borne in a closed ovary, and flowers are perfect or imperfect.
   b. Angiosperms have true flowers, but gymnosperms have strobili (cones).
   c. Double fertilization takes place in angiosperms; single fertilization takes place in gymnosperms.
   d. In gymnosperms, the developing embryo

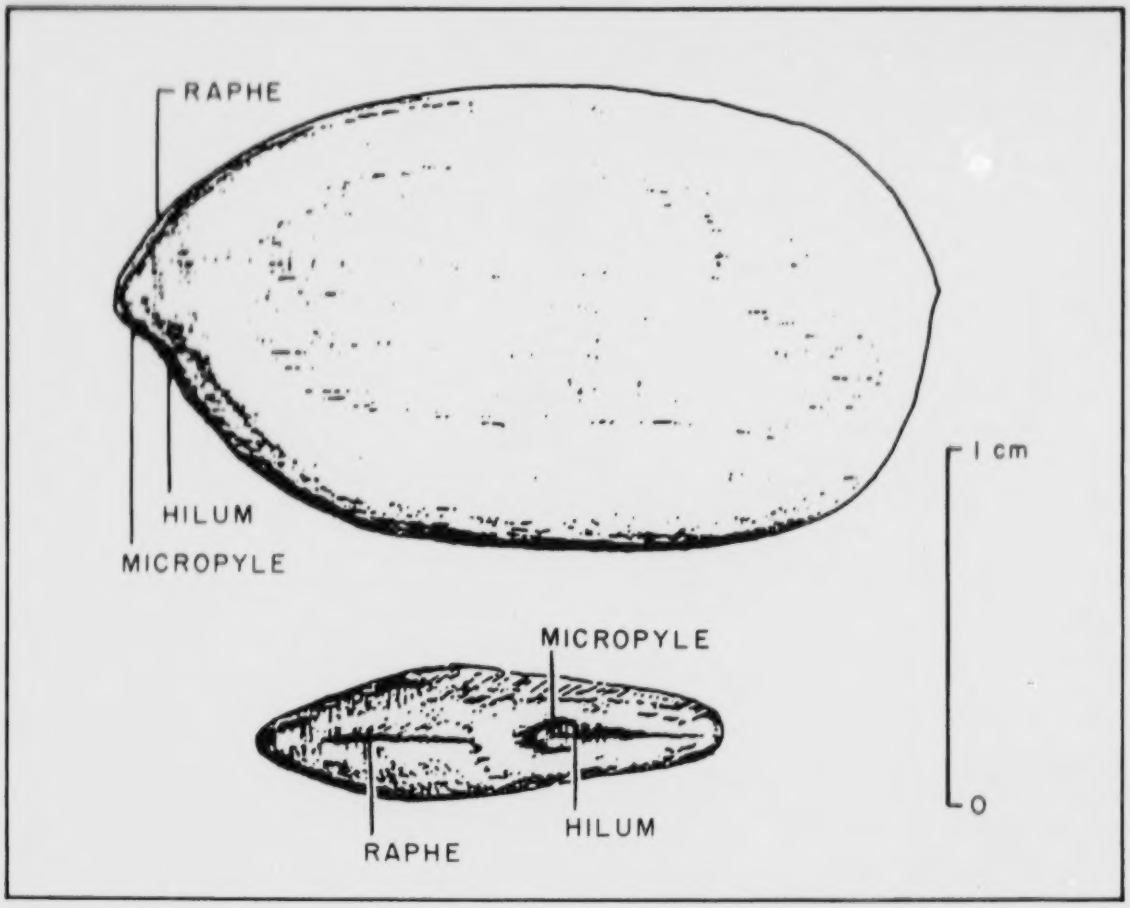

Figure 3.–*External morphology of a typical legume seed of* Schizolobium parahybium *(adapted from Triviño and others 1990).*

is nourished by the haploid female gametophyte; in angiosperms, it is nourished from either diploid cotyledons, hypocotyl of the embryo, triploid endosperm, or diploid nucellar material.

G. Seed and Fruit Development

1. **Physical development**

a. Angiosperms

(1) Pollination and fertilization trigger:

(a) formation of embryo and endosperm

(b) cell divisions and enlargements

(2) Legumes have:

(a) a simple pistil with a superior ovary having one cavity (locule) (fig. 3)

(b) seedcoats composed of histologically dense cuticle, radial columnar cells, sclerenchymatous cells, lignin, and osteosclereid cells

(3) Structural terms relating to the seedcoat are defined as follows (fig. 4):

(a) **cuticle**–waxy layer on outer walls of epidermal cells

(b) **lignin**–organic component of cells associated with cellulose

(c) **light line**–continuous thin layer of wax globules

(d) **osteosclereid**–bone-shaped sclerenchyma

(e) **palisade cells**–elongated cells perpendicular to the coat surface

(f) **parenchyma**–undifferentiated, live cells

(g) **sclerenchyma**–thick, lignified cells

b. Gymnosperms–Many conifers flower and ripen seeds in one growing season, some require two seasons, and a few require three seasons.

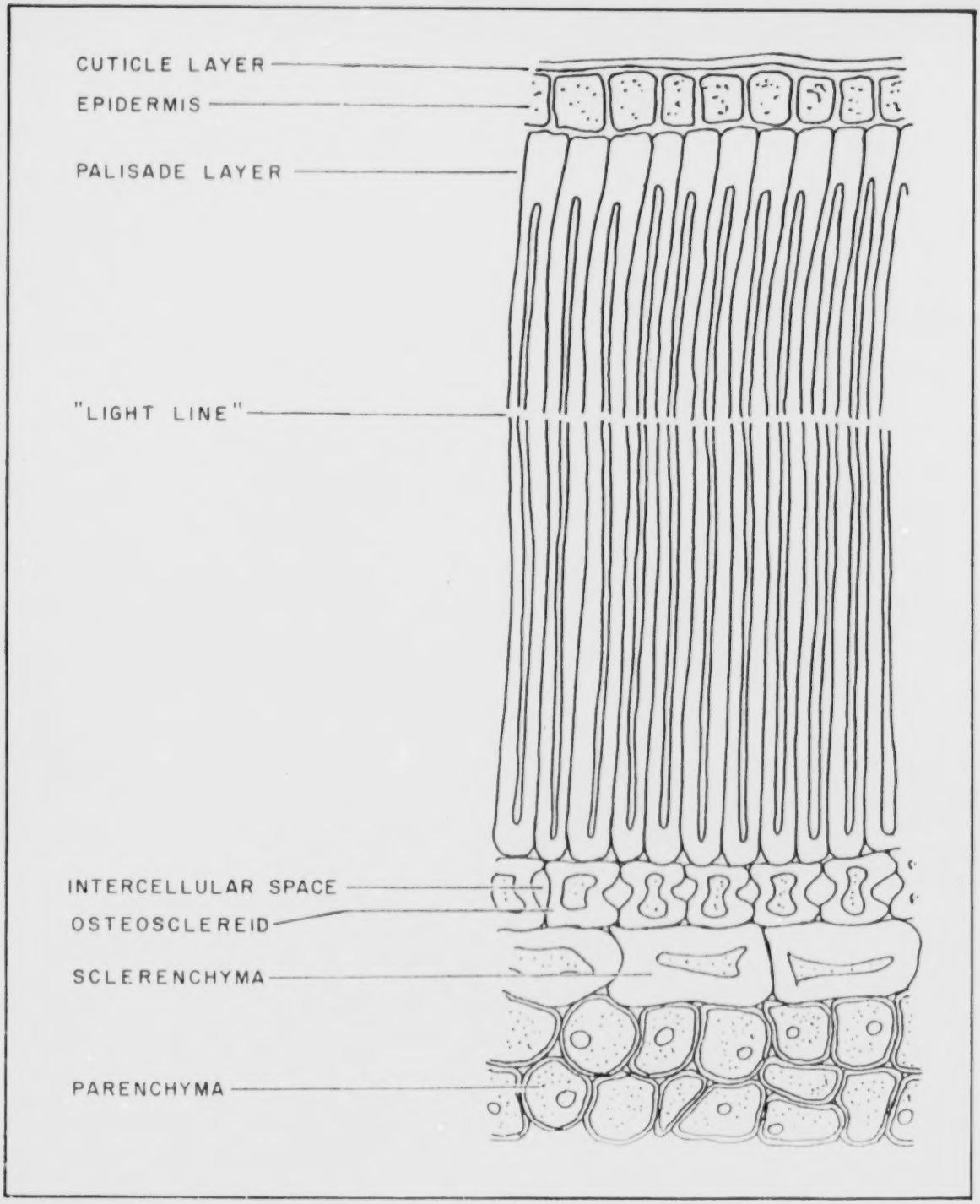

Figure 4.—*Partial section through the seedcoat of a hard seed (legume).*

2. **Physiological development**
   a. Moisture content increases rapidly after fertilization and decreases at maturity.
   b. Hormone contents are higher where meristematic activity is greater.
   c. Metabolic changes are many; simple sugars, fatty acids, and amino acids are converted to proteins, oils, and lipids.
3. **Classification of mature fruits** (table 1)

H. Sources

For additional information, see Dogra 1983; Hardin 1960; Hartmann and others 1983, chap. 3, p. 59–65; Krugman and others 1974; Willan 1985, p. 7–10, 13–15.

II. Seed Dormancy

A. Introduction

Once seeds have matured, survival of the species requires that they germinate at a time and place favorable for growth and survival of the seedlings. The mechanism that prevents germination at undesirable times is called dormancy. The mechanics of seed dormancy must be known before nursery practices for overcoming dormancy can be developed to ensure timely germination and uniform growth of the seedlings.

B. Objectives

1. Describe the different types of seed dormancy.
2. Discuss methods for overcoming seed dormancy, both for germination testing and for nursery operations.

C. Key Points

The following points are essential to understanding seed dormancy:

1. To a large degree, dormancy is under genetic control.
2. Environmental conditions during seed maturation can influence the degree of dormancy.
3. Seeds can have more than one type of dormancy mechanism.
4. Postharvest environment can create secondary dormancy.
5. The distinction between "dormancy" and "delayed germination" is not always clear.
6. The least severe treatment to overcome dormancy should be tested first to avoid damage to the seeds.

D. Definition of Terms (Bonner 1984a)

1. **Afterripening** – physiological process in seeds after harvest or abscission that occurs before, and is often necessary for, germination or resumption of growth under favorable environmental conditions.

Table 1.—*Common fruit types for woody trees (adapted from Hardin 1960)*

Description	Type	Example
Simple Fruit (product of single pistil)		
Dehiscent walls (splitting naturally)		
Product of one carpel		
Dehiscing by one suture	Follicle	*Zanthoxylum*
Dehiscing by two sutures	Legume	*Acacia, Prosopis, Robinia*
Product of two or more carpels	Capsule	*Eucalyptus, Populus*
Walls indehiscent (not splitting naturally)		
Exocarp fleshy or leathery		
Pericarp fleshy throughout	Berry	*Vaccinium, Diospyros*
Pericarp heterogeneous		
Exocarp leathery rind	Hesperidium	*Citrus*
Exocarp fleshy		
Endocarp a "stone"	Drupe	*Prunus, Vitex, Tectona*
Endocarp cartilaginous	Pome	*Malus, Crataegus*
Exocarp dry (papery, woody, or fibrous)		
Fruit winged	Samara	*Triplochiton, Terminalia, Acer*
Fruit without wings		
One-loculed ovary; thin wall; small seed	Achene	*Platanus, Cordia*
Several-loculed ovary; thick wall; large seed	Nut	*Quercus*
Compound Fruit (product of multiple pistils)		
Pistils of a single flower	Aggregate	*Magnolia*
Pistils from different flowers (inflorescence)	Multiple	*Platanus*

2. **Dormancy** – a physiological state in which a seed disposed to germinate does not, even in the presence of favorable environmental conditions.
3. **Chilling** – subjection of seeds to cold and moisture to induce afterripening.
4. **Prechilling** – cold, moist treatment applied to seeds to hasten afterripening or to overcome dormancy before sowing in soil or germinating in the laboratory.
5. **Pretreatment** – any kind of treatment applied to seeds to overcome dormancy and hasten germination.
6. **Scarification** – weakening of seedcoats, usually by mechanical abrasion or by brief soaks in strong acids, to increase their permeability to water and gases or to lower their mechanical resistance to swelling embryos.
7. **Stratification** – placing seeds in a moist medium, often in alternate layers, to hasten afterripening or to overcome dormancy; commonly applied to any technique that keeps seeds in a cold, moist environment.
8. **Delayed germination** – a general term applied to seeds that do not germinate immediately but are not slow enough to be described as dormant.

E. Types of Dormancy
1. **Seedcoat (or external) dormancy**
   a. Impermeability to moisture or gases; e.g., *Acacia, Prosopis, Robinia,* and other legumes
   b. Mechanical resistance to swelling embryo; e.g., *Pinus* and *Quercus.*
2. **Embryo (or internal) dormancy**
   a. Inhibiting substances; e.g., *Fraxinus, Ilex,* and *Magnolia*
   b. Physiological immaturity; e.g., *Juniperus virginiana*
3. **Morphological dormancy** results from the embryo not being completely developed; e.g., *Ilex opaca,* some *Fraxinus* spp., and *Pinus* spp.
4. **Secondary dormancy** results from some action, treatment, or injury to seeds; e.g., *Pinus taeda* being exposed to high temperatures and moisture during storage.
5. **Combined dormancy** results from two or more primary factors, such as seedcoat dormancy and embryo dormancy, e.g.; *Tilia.*
6. **Double dormancy** results from embryo dormancy in both the radicle and epicotyl; e.g., *Prunus.*

F. Overcoming Dormancy
1. **Seedcoat dormancy** – Treatment must increase moisture uptake and gas exchange and ease radicle emergence.
   a. Cold water soak – Soak seeds in water at room temperature for 24 to 48 hours.
   b. Hot water soak – Bring water to a boil, put seeds in, remove from heat, and allow to stand until water cools.
   c. Hot wire – Use a heated needle or electric woodburner to burn a small hole through the seedcoats.
   d. Acid treatment – Pour a strong mineral acid over the seeds and mix. (Sulfuric acid is preferred.) Remove seeds after a time determined by trials with samples, usually 15 to 60 minutes, and wash thoroughly to remove acid.
   e. Physical scarification – Crack or break the hard seedcoats.
      (1) Use hand methods (nicking).
      (2) Use mechanical methods for large-scale operations.
2. **Embryo dormancy** – Treatment must overcome physiological barriers within the seeds.
   a. Stratification (chilling, prechilling) – Refrigerate fully imbibed seeds at 1 to 5 °C for 1 to 6 months (table 2).
      (1) Imbibition is completed.
      (2) Enzyme systems are activated.
      (3) Storage foods change to soluble forms.
      (4) Inhibitor/promoter balances change.
   b. Incubation/stratification – For some species, provide short, warm incubation (15 to 20 °C), followed by cold stratification.
   c. Chemical treatment
      (1) Hydrogen peroxide – Soak for 48 hours in 1-percent solution (e.g., *Pseudotsuga menziesii*).
      (2) Citric acid – Soak for 48 hours in 1-percent solution, followed by 90-day stratification (e.g., *Juniperus, Taxodium distichum*).
      (3) Gibberellins
      (4) Ethylene
   d. Light – Dormancy is overcome by the red/far-red mechanism.

G. Significance
1. **Survival strategy** – Dormancy allows germination during favorable environmental conditions.
2. **Genetic factor** – Dormancy in many seeds is under genetic control.
3. **Multiple causes** – Many species have probably evolved with more than one dormancy mechanism.

Table 2. – *Recommended prechill periods for nursery sowing of some pines of the Southern United States (Bonner 1991b)*

Pine species	Normal sowing*		Early sowing†	Seed conditions	
	Fresh seed	Stored seed		Deep dormancy‡	Low vigor
	Prechill *(Days)*				
Pinus strobus	30–60	60	60–90	60–90	30
P. taeda	30–60	30–60	60	60–90	20–30
P. palustris	0	0	...§	0–15	0
P. rigida	0	0–30	...	...	...
P. serotina	0	0–30	...	...	...
P. clausa					
var. *immuginata*	0–15	0–21	...	...	...
var. *clausa*	0	0	...	...	...
P. echinata	0–15	0–30	15–30	30–60	0
P. elliottii					
var. *elliottii*	0	0–30	...	15–30	0
var. *densa*	30	0–30	...	...	...
P. glabra	30	30	...	...	...
P. virginiana	0–30	30	30	...	...

*Spring sowing when mean minimum soil temperature at seed depth is at least 10 °C.
†Early sowing when soil temperatures at seed depth may be below 10 °C.
‡Dormancy demonstrated by paired tests or past performance of the seedlot.
§Conditions not encountered with this species.

4. **Environmental influence** – Weather conditions during maturation may increase the degree of dormancy.

H. Sources
For additional information, see Khan 1984; Krugman and others 1974; Murray 1984b; Nikolaeva 1967; Willan 1985, p. 17–19, chap. 8.

III. Germination

A. Introduction
The goals of seed technology are successful germination and seedling establishment. The two major considerations are the physiology of the seed and the condition of the environment. In the two preceding sections, seed maturation and dormancy were considered. In this section, environmental factors and how they control germination through their interactions with seed biology will be examined.

B. Objectives
1. Describe the two types of germination and their importance in woody plants.
2. Review environmental requirements for germination.
3. Review physiological changes within seeds that lead to germination.
4. Discuss how seed physiology and environmental factors interact in germination.

C. Key Points
The following points are essential to understanding germination:
1. The two types of germination are epigeous and hypogeous.
2. Moisture availability is the primary factor controlling germination.
3. The effects of temperature and light on germination are strongly related.
4. Constant and alternating temperature regimes may lead to similar total germination, but germination is usually faster under alternating regimes.
5. As germination begins, the key to internal processes is the change from insoluble to soluble metabolites. Details of such metabolism are beyond the scope of this course.

D. Types of Germination
1. **Epigeous (epigeal)** germination occurs when cotyledons are forced above the ground by elongation of the hypocotyl (fig. 5); e.g., *Pinus, Acacia, Fraxinus,* and *Populus.*
2. **Hypogeous (hypogeal)** germination occurs when the cotyledons remain below ground while the epicotyl elongates (fig. 6); e.g., *Juglans, Quercus,* and *Shorea.*
3. In *Prunus,* both types of germination may be found.

E. Environmental Requirements for Germination
The four environmental requirements for germination are moisture, temperature, light, and gases.
1. **Moisture**
a. Imbibition is usually considered the first step in germination; thus, avail-

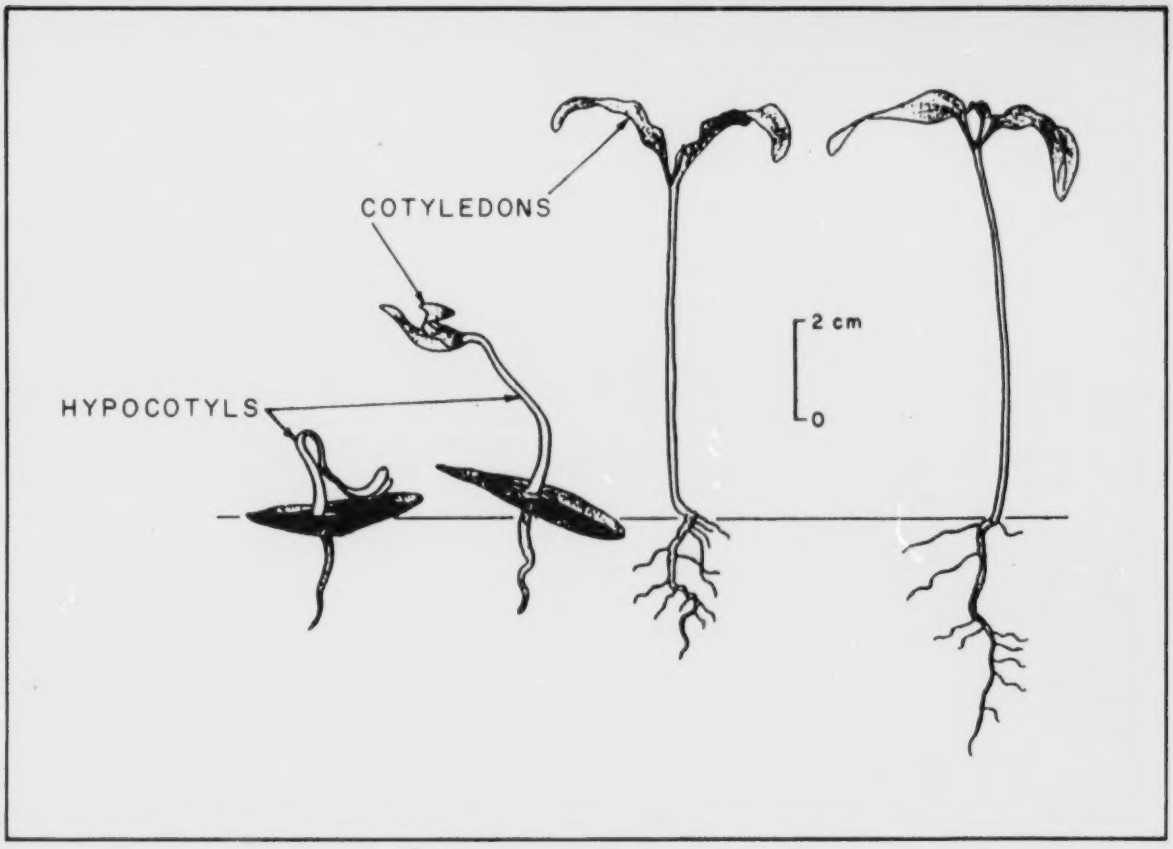

Figure 5 . – *Epigeal germination sequence of* Fraxinus *spp. (adapted from Bonner 1974).*

ability of moisture is the first requirement for germination.

b. Uptake typically occurs in the following three phases:
   (1) Rapid initial phase, mainly physical
   (2) Extremely slow second phase
   (3) Rapid third phase that occurs as metabolism becomes very active
c. The first phase is imbibitional.
d. A minimum state of hydration is needed.
e. Minimal requirements for germination are frequently studied with osmotic solutions of mannitol or polyethylene glycol.
   (1) The best germination may occur at slight moisture stress (0.005 to 0.500 bars).
   (2) Even slightly lowered water potentials will slow, but not stop, germination.
   (3) Critical levels of water potential vary by species.

2. **Temperature**
   a. It is difficult to separate the effects of temperature from those of light and moisture.
   b. For woody plants, germination usually occurs over a wide range of temperatures.
   c. The upper temperature limit is around 45 °C.
   d. The lower limit is around 3 to 5 °C because germination processes will occur near freezing.
   e. Optimum temperatures vary little:
      (1) For Temperate Zone species, alternating regimes of 20 °C (night) and 30 °C (day) have proved best for many species.
      (2) For tropical species, although few critical studies are available, constant temperatures may be best for some; e.g., *Azadirachta indica*, 25 °C; *Bombax ceiba*, 25 °C; *Eucalyptus camaldulensis*, 30 °C; *Leucaena*

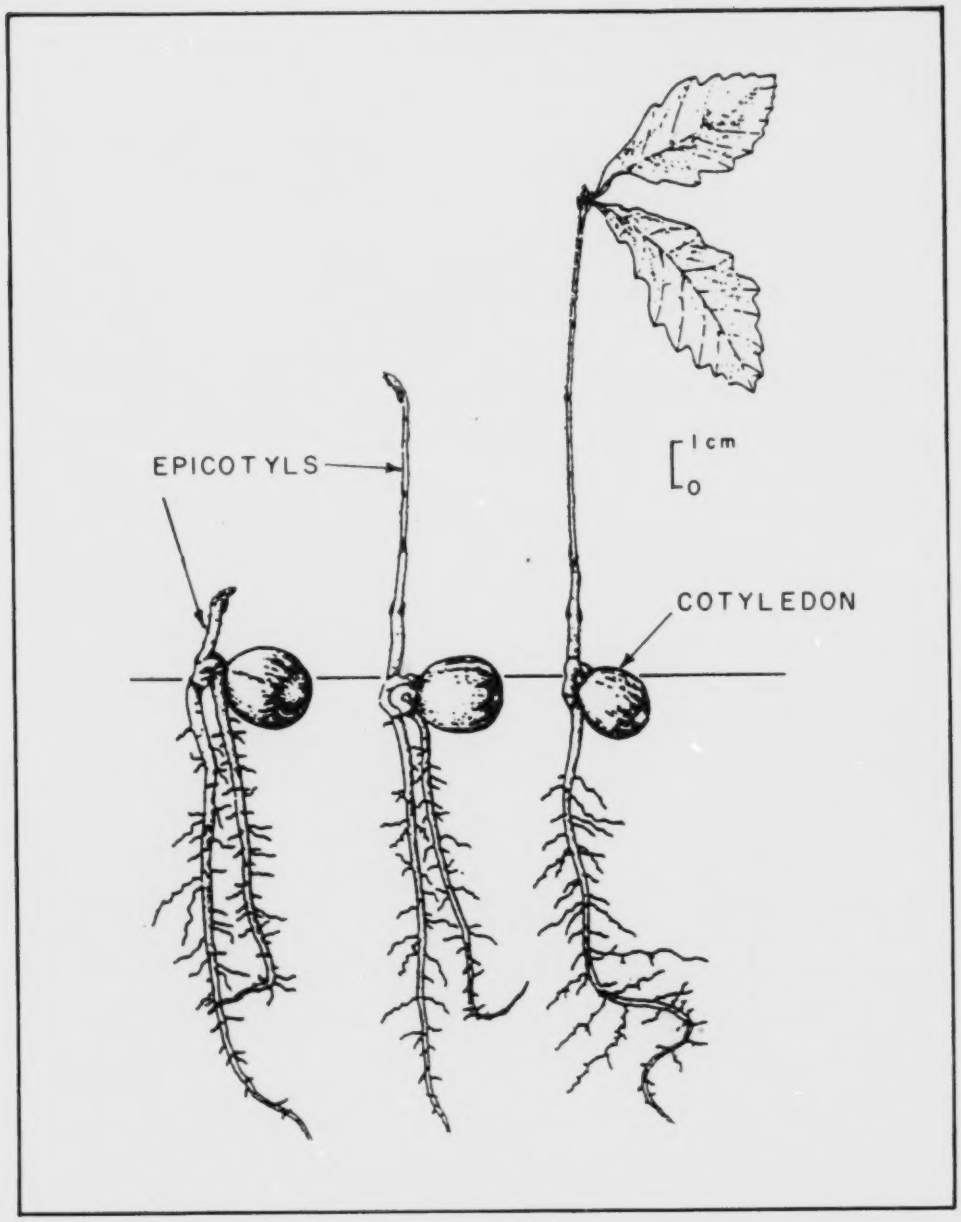

Figure 6.—*Hypogeal germination sequence of* Quercus *spp. (adapted from Olson 1974).*

leucocephala, 30 °C; *Prosopis cineraria*, 30 °C; and *Tectona grandis*, 30 °C. Other species do as well or even better under alternating temperatures; e.g., *Acacia* spp., *Cedrela* spp., and tropical pines.

3. **Light**
   a. Light stimulates germination of many tree seeds but is necessary for few.
   b. Phytochrome is a pigment involved in the photocontrol of germination.
   c. Minimal light levels in germination testing should be 750 to 1,250 lux.
4. **Gases**
   a. Respiration requires a certain supply of oxygen, and the carbon dioxide produced must be removed.
   b. Some species germinate well in anaerobic conditions.
   c. Oxygen uptake patterns in seeds are similar to those of moisture.
   d. Many aspects of the influences of gases on germination need to be studied.

F. Internal Physiological Changes
   1. **Structural changes** – Imbibition is a precursor to necessary metabolism.
   2. **Enzymes** – Some systems are present in dry seeds; others are synthesized as imbibition proceeds.
   3. **Reserve food mobilization** – Generally, insoluble forms (carbohydrates, lipids, and proteins) are converted to soluble forms (in some ways a reverse of maturation trends).
   4. **Nucleic acids** – These compounds are essential for the formation of new enzymes.
   5. **Translocation** – The movement of materials within the embryo is crucial.

G. Sources

For additional information, see Bonner 1972, Mayer and Poljakoff-Mayber 1975, Murray 1984b, Stanwood and McDonald 1989, Willan 1985.

Collection

I. Genetics and Seed Source

A. Introduction

Seed quality involves both the genetic and the physiological quality of seeds. In this section, the general principles and methods for selection of seed source and improvement of seed quality through genetic selection are presented. Genetic improvement of seed quality is based on the seeds' ability to produce trees that are genetically well suited to the sites where planted and for the products desired. In later sections, physiological quality of seeds will be considered. Good seeds are those that have both high physiological quality and genetic suitability.

B. Objectives

1. Recognize the importance of seed origin (provenance) and recommend general rules for seed movement.
2. Review the advantages and disadvantages of exotic tree species and interspecific hybrids for tree improvement.
3. Define factors that must be considered when a tree improvement program is initiated.
4. Identify the conditions required for genetic improvement of tree seeds (genetic gain concept).
5. Distinguish between a minimum initial strategy of genetic improvement and a maximum long-term strategy.
6. Identify some terms and concepts of new biotechnology for genetic improvement.

C. Key Points

The following points are essential to understanding seed source and genetic improvement:

1. A successful tree improvement program should not be tried in another country or region without considering desired products and available sites.
2. Knowledge of phenotype and genotype is necessary to understand genetic improvement of trees.
3. The genetic gain equation explains the advantages of one improvement method over another.
4. Genetic gains can be obtained from selections among species, provenances within species, and/or trees within provenances.
5. The primary risk of using exotics or nonlocal provenances is planting on unsuitable sites.
6. Test plantings are the only sure method to determine genetic quality of seeds.
7. Without results of test plantings, the safest rule is to use seeds from phenotypically selected stands or trees in the local provenance for native species or land race for exotic species.
8. The seed orchard concept has two parts—the breeding program and the production program.
9. Seed orchard breeding programs involve progeny tests and selection for the next advanced generation of genetic improvement.
10. Seed orchard production programs are managed to maximize seed production through protection and cultural treatments.

D. Tree Improvement

1. **Tree improvement** is the development and application of genetically improved trees and intensive cultural practices to enhance forest productivity through artifical regeneration.
2. **Tree improvement programs** are plans of action to bring about desired objectives. The following factors should be considered when a tree improvement program is initiated:
   a. Products desired
   b. Sites to be regenerated
   c. Adaptation to the planting sites
   d. Conservation of forest gene resources

E. Strategies for Genetic Improvement

1. **Genetic gain**
   a. Genetic improvement (genetic gain) is accomplished by:
      (1) Having a population of trees with genetic differences
      (2) Selecting the genetically desirable trees
   b. The amount of genetic gain *(R)* to be captured from phenotypic selection of parent trees for a particular trait is:

      $$R = i\, V_P\, h^2$$

      where i = the intensity of selection
      h^2 = the heritability of the trait
      V_P = the amount of phenotypic variation.
   c. Gain can be captured from selection among species (R_S), selection among provenances within species (R_P), or selection among individual trees within provenances (R_I). The total gain (R_T) is the sum:

      $$R_T = R_S + R_P + R_I$$

2. **Species selection**
   a. Species-site studies are needed.
   b. Exotic tree species should be used sparingly.

c. Interspecific hybridization can be used to obtain valuable traits.

3. **Seed source**
   a. Provenance refers to where the mother trees were growing and the seeds were collected. Seed source is the same as provenance. Origin is where the original progenitors were growing in natural forests and where their genetic characteristics were developed through natural selection.
   b. "Local" sources should be used until provenance test results are available.
   c. Purposes of provenance tests include:
      (1) Mapping patterns of geographic genetic variation
      (2) Delineating provenance boundaries
      (3) Determining the best provenances
   d. The general results of provenance testing are:
      (1) Wide seed transfer is safer near the center of a species' range than near its edge.
      (2) Where environmental gradients are steep, movement of material must be restricted.
      (3) Provenances from harsh climates (cold or dry) grow more slowly.

4. **Improvement strategies**
   a. The initial strategies for a new program are:
      (1) Collect available information
      (2) Select among indigenous tree species
      (3) Select seed production areas within the "local" seed sources near the planting site
      (4) Remove phenotypically inferior trees from seed production areas
   b. The long-term strategies for maximum and continued gains are:
      (1) Collect all existing information
      (2) Select several species for the program
      (3) Conduct provenance tests
      (4) Select the phenotypically "best" trees
      (5) Establish a first-generation seed orchard
      (6) Test progeny
      (7) Remove genetically poor trees
      (8) Select the best individuals for a second-generation seed orchard
      (9) Test the progeny of these second-generation selections
   c. New strategies for genetic improvement are:
      (1) Gene transfer
      (2) Selection of cells in a cell-suspension culture
      (3) Fusion of protoplasts (without cell walls)
      (4) Somaclonal variation

F. The Seed Production Program
The production program may be combined with the breeding program or may be kept separate. The objective of a seed production program is to produce sufficient quantities of genetically high-quality seeds to meet seed needs.

1. **Seed Production Areas** (SPA's)
   a. Existing stands can be managed for production of seeds.
   b. SPA's are used on an interim basis.
   c. SPA's can utilize superior provenances.
   d. SPA's can provide seeds for minor species.
   e. The genetic quality of seeds is improved by:
      (1) Removing undesirable trees
      (2) Establishing a pollen dilution zone
   f. Seed production is increased by:
      (1) Thinning the stand
      (2) Fertilizing
      (3) Establishing access roads

2. **Seed orchards**—A seed orchard is a collection of selected trees established and grown together under intensive management for production of genetically improved seeds.
   a. There are two types of orchards:
      (1) Seedling seed orchards
      (2) Clonal seed orchards
   b. The genetic quality of seeds is increased by:
      (1) Reducing inbreeding
      (2) Establishing a pollen dilution zone
      (3) Separating provenances into different orchards
   c. Seed production from orchards can be increased by:
      (1) Choosing good soil and climatic conditions
      (2) Spacing wide enough for full crowns
      (3) Fertilizing
      (4) Irrigating
      (5) Subsoiling
      (6) Protecting from insects
      (7) Protecting flowers from late spring freezes (cold water irrigation)
      (8) Ensuring supplemental mass pollination

G. Sources
For additional information, see Burley and Styles 1976, Khosla 1982, Nienstadt and

Snyder 1974, Rudolf and others 1974, Wright 1976, Zobel and Talbert 1984, Zobel and others 1987.

II. Production

A. Introduction

Most tree-planting programs are begun by collecting seeds from in-country sources, both natural stands and plantations. To plan these collections effectively, seed managers should understand the factors that affect tree seed crops and generally know what seed yields may be expected. With this basic information, opportunities may arise to stimulate seed production in key areas, such as seed orchards or managed seed stands.

B. Objectives

1. Recognize the problem of periodicity of seed production in trees.
2. Learn how environmental factors affect seed production.
3. Learn how seed production can be stimulated in trees.

C. Key Points

The following points are essential to understanding seed production:

1. Many tree species bear good crops in cycles.
2. Production is less frequent in high latitudes and high altitudes and among heavy predator populations.
3. Environmental factors influence flower production, pollination, and seed maturation.
4. Several options are available to stimulate seed production.
5. Except for seed orchards of a few species, production data are extremely variable.

D. Periodicity of Seed Crops

1. **Temperate species**
   a. Many conifers bear in cycles.
   b. Many angiosperms produce good seed crops every year.
   c. As latitude or altitude increases, the interval between good crops and the frequency of crop failure increase.
2. **Tropical species**
   a. Periodicity may depend on wet/dry cycles.
   b. Some species (e.g., *Tectona grandis*) usually flower each year. Other species (e.g., *Pinus kesiya*, *Cassia siamea*, *Cupressus lusitanica*, and *Delonix regia*) produce good crops most years.
   c. Dipterocarps in Malaysia bear irregular heavy seed crops at 1- to 6-year intervals.
   d. Some *Eucalyptus* species have large crops more regularly when grown in plantations.
3. **Genetics**—Fecundity is an inherited trait.
4. **Documentation**—There are few detailed studies and data.

E. Effects of Environments During Flowering

1. **Temperature**
   a. During hot summers, trees usually produce heavy floral bud formation.
   b. Late freezes can destroy flowers.
   c. The combination of hot summers and late freezes suggests that orchards should be moved to warmer climates (also to escape insects).
2. **Light** has not been studied extensively. In the Northern Temperate Zone, southern and western sides of crowns have the heaviest flower and fruit crops.
3. **Photoperiod** does not appear to have a direct effect on trees.
4. **Moisture** affects flowering through:
   a. Drought
   b. Excessive rain during pollination
5. **Mineral nutrients**—The balance of nitrogen and phosphorus can affect flowering.
6. **Biotic agents**—Insects, birds, mammals, and micro-organisms can destroy flowers. These agents are very common in the following tropical tree species:
   a. *Triplochiton scleroxylon;* attacked by *Apion ghanaense* (weevil)
   b. *Tectona grandis;* attacked by *Pagyda salvaris* larvae
   c. *Pinus merkusii;* attacked by *Dioryctria* spp. (cone worms)

F. Pollination Agents

1. **Wind pollination** occurs among all conifers and most Temperate Zone hardwoods.
   a. Wind pollination requires:
      (1) Lots of pollen
      (2) Pollen shed coinciding with receptivity
      (3) Relatively close spacing of plants
      (4) Good weather—low rainfall, low humidity, and good winds
   b. Supplemental mass pollination (SMP) has been used in United States southern pine orchards.
   c. Contamination in orchards is a concern.
2. **Animal pollination**
   a. Insects and bats pollinate temperate and tropical hardwoods.
   b. Animal pollination is usually common in tropical forests with:

(1) High species diversity and wide spacing
(2) Abundant foliage to filter out pollen
(3) High humidity and frequent rainfall
(4) Absence of strong flowering stimuli
(5) Abundant animal vectors

G. Stimulation of Flowering
Flowering can be stimulated by several management practices.
1. **Fertilizing**:
   a. Use primarily nitrogen and phosphorous, and sometimes potassium.
   b. Irrigation at the same time may also help.
   c. Hardwoods may react favorably; e.g., *Acer, Fagus,* and *Juglans*, but results have been inconsistent.
2. **Girdling and other wounding** can produce "stress crops."
   a. Girdling inhibits downward translocation of carbohydrates.
   b. Some hardwoods also react favorably.
3. **Thinning**—The benefits of thinning are apparent 3 to 4 years after treatment.
4. **Growth regulator treatment**—Gibberellins (GA) application to conifers is the most common.
   a. A water-based spray is best.
   b. A GA 4/7 mixture is most effective.
   c. Both pollen and seed cones are induced.
   d. Sprays are applied at the time of bud determination.
   e. The mode of action is still unknown.
   f. Treatments are most successful when applied with girdling, root pruning, or moisture stress.
5. **Supplemental mass pollination** (SMP)—This technique is used in pine orchards in the Southern United States.

H. Postfertilization Problems
Postfertilization problems include insect damage to cones, drought, cone drop, and high winds.

I. Sources
For additional information, see Franklin 1982; Owens and Blake 1985; Rudolf and others 1974; Whitehead 1983; Willan 1985, chap. 3; Zobel and Talbert 1984.

III. Collection Operations

A. Introduction
Successful collection of tree seeds is usually the result of quite detailed early planning. Ample time must be allowed to plan an efficient and practical collection strategy and to assemble the resources necessary for its implementation. Key elements include a good estimate of crop size, proper equipment, and a well-trained crew. Comprehensive collections for research will almost certainly require more detailed planning than routine bulk collections and may require a lead time of 1 to several years depending on the circumstances.

B. Objectives
1. Identify simple techniques for seed crop estimation.
2. Determine factors that should be considered when collections are planned.
3. Understand the importance of documentation.

C. Key Points
The following points are essential for planning collection operations:
1. The best seed sources available must be selected.
2. Good planning requires advance estimates of the seed crop and, at a later date, estimates of seed yield per fruit.
3. Planning for large collections must include choice of personnel, training, transportation, collection equipment, safety of workers, labeling of seedlots, description of sites and stands, etc.

D. Seed Source
Seed source includes the following considerations:
1. **Origin**—the natural stand location of the original mother tree.
2. **Provenance**—the place where mother trees that produced the seeds are growing. (Same as seed source.)
3. **Land race**—exotics that adapt over time to provide improved sources.
4. **Seed zone maps** should be developed for all important species.

E. Estimating Seed Crops
Seed crop estimates are always valuable to the collector, especially in years when seeds are in short supply. Good crop estimates help to stretch the available crews and equipment. Seed crops can be estimated by the following five methods:
1. **Flower counts**
2. **Immature fruit and seed counts**
3. **Fruit counts on standing trees**—This method includes total counts and crown sampling.
4. **Rating systems**
5. **Cross-section seed counts** (table 3).

F. Planning Considerations
The steps of planning a collection are:
1. **Define the objectives.**

Table 3.—*Sound seed yield per cone for four* Pinus *species as estimated from the number of sound seeds exposed when cones are bisected longitudinally (Derr and Mann 1971)*

Sound seeds exposed	*P. palustris* (Louisiana)	*P. taeda* (Louisiana)	*P. elliottii* (Louisiana)	*P. elliottii* (Georgia-Florida)	*P. echinata* (Virginia)
	Sound seeds per cone				
2	23	31	20	31	12
4	35	44	35	50	22
6	47	57	50	69	31
8	59	70	65	87	41
10	71	83	80	106	51
12	83	96	95	124	60
14	95	109	110	143	70

2. **Gather background data:**
   a. Search the literature.
   b. Officially contact the appropriate forest services early.
   c. Collate and summarize all information.
   d. Do field reconnaissance.
   e. Determine the number of personnel needed.
3. **Collect field data:** Information for relocation of the site in future years:
   a. Locality, including latitude and altitude
   b. Aspect, slope, climate, soils, and associated species
   c. Individual tree descriptions
   d. Herbarium specimens
   e. Other data and notes
   f. Security and labeling
4. **Plan the itinerary:**
   a. Reach the collection region well in advance of the proposed date.
   b. Organize the sequence of operations.
   c. Make the schedule flexible.
5. **Organize equipment permits and transportation:**
   a. Specify equipment to be used.
   b. Identify applicable government regulations.
   c. Use care between the collection of the seeds and their arrival in the seed laboratories.

G. Collection Equipment—A Comprehensive List
The following items are necessary for most collection operations:

1. **Administrative items**
   a. Movement approvals
   b. Collection authorities
   c. Radio transmission permits
   d. Drivers' licenses
   e. Firearm permits
   f. Facilities for purchasing stores; e.g., gasoline (petrol) and oil
2. **Literature**
   a. Road, topographic, and soil maps to cover the collection route itinerary
   b. Literature on the genera and species to be collected
3. **Collection equipment**
   a. Notebooks, recording forms, pens, and pencils
   b. Binoculars
   c. Markers; e.g., colored plastic ribbon
   d. Camera and accessories
   e. Tree-measuring instruments; e.g., diameter tape, height-measuring instrument, and length tape
   f. Soil sampler, pH testing kit, and soil charts
   g. Compass
   h. Altimeter
   i. Hand lens
   j. Large collecting sheets; e.g., 4 by 4 m, heavy plastic or canvas
   k. Small collecting sheets
   l. Seed bags of various sizes
   m. Large grain bags for dispatching seeds
   n. Cutting equipment
   o. Safety gear
   p. Weatherproof tags
   q. Tags for botanical specimens
   r. Plant presses
   s. Papers to dry specimens
   t. Plastic bags
   u. Specimen bottles with preservative fluid
   v. Containers for soil samples
   w. String

H. Sources
For additional information, see Barner and Olesen 1984; Bramlett and others 1977; Doran and others 1983; Ontario Ministry of Natural Resources 1983; Willan 1985, chaps. 3, 4, 5 + appendices 1, 5, 6.

IV. Maturity

A. Introduction

Choosing good stands and trees for seed collection means nothing if fruit or seed maturity cannot be easily identified on the trees by unskilled workers. If seeds are disseminated immediately at maturity, workers must know how much in advance of maturity seeds can be collected without collecting seeds that will not germinate. If predators inflict large losses on mature seed crops, a similar problem exists. Good maturity indices are often the key to successful collection.

B. Objectives

1. Learn the common indices of maturity employed in tree seed collections.
2. Understand how these techniques can be adapted for new species.

C. Key Points

The following points are essential to recognizing seed maturity:

1. Seed moisture content is very important, but direct measurement in the field is impractical; indirect estimates may be substituted.
2. Color changes are the most common indices.
3. Chemical indices are possible but impractical.
4. Artificial maturation of immature seeds is an option for some species.

D. Successful Collection

The following points are essential to successful collection:

1. **Biological ideal** – to collect at the peak of physiological maturity
2. **Practical collection** – In most collection operations, one may:
   a. Collect seeds from the ground
   b. Collect fruits or seeds from logging operations
   c. Collect mature fruits from standing trees
   d. Collect fruits from standing trees well in advance of maturity and ripen the seeds artificially

E. Collection after Dissemination

Some seeds can be collected after dissemination. These seeds are primarily large, single-seeded fruits; e.g., species of *Quercus* and *Carya*. However, the first seeds to fall are usually bad. Workers must quickly collect the seeds before animals eat them.

F. Other Collection Strategies

Other collection strategies require determination of maturity.

G. Maturity Indices

Maturity indices include physical and chemical characteristics.

1. **Physical characteristics** include:
   a. Color change
   b. Moisture content
      (1) There are three trends during ripening:
         (a) In dry, orthodox seeds and fruits, moisture decreases slowly as seeds mature.
         (b) In pulpy, orthodox fruits, moisture decreases at first, then increases.
         (c) In recalcitrant seeds, moisture increases early, then slightly decreases.
      (2) Moisture content is related to protein synthesis.
      (3) Moisture content can be measured directly by oven methods; that is, cut cones, large fruits, or seeds; weigh; dry for 17 hours at 103 °C; and then weigh again.
      (4) Specific gravity is usually discussed separately, but it really is just an estimate of moisture content (table 4; figs. 7 and 8). Specific gravity has been measured in:
         (a) Conifers (commonly used for *Pinus*)
         (b) *Quercus* (inconsistent results)
         (c) Other angiosperms (little success)
   c. Other physical indices include:
      (1) Acorn cup release in *Quercus*
      (2) Flex of *P. strobus* cones
      (3) White, brittle embryo in *Fraxinus* and other genera
      (4) Embryo size (minimum percentage of embryo cavity)

Table 4. – *Cone specific gravity values that indicate seed maturity in some conifers*

Species	Specific gravity	Reference
Abies grandis	0.90	Pfister 1967
Cunninghamia lanceolata	0.95	Jian and Peipei 1988
Pinus elliottii	0.95	Barnett 1976
P. merkusii	1.00	Daryono and others 1979
P. palustris	0.90	Barnett 1976
P. strobus	0.90	Bonner 1986a
P. taeda	0.90	Barnett 1976
P. virginiana	1.00	Fenton and Sucoff 1965

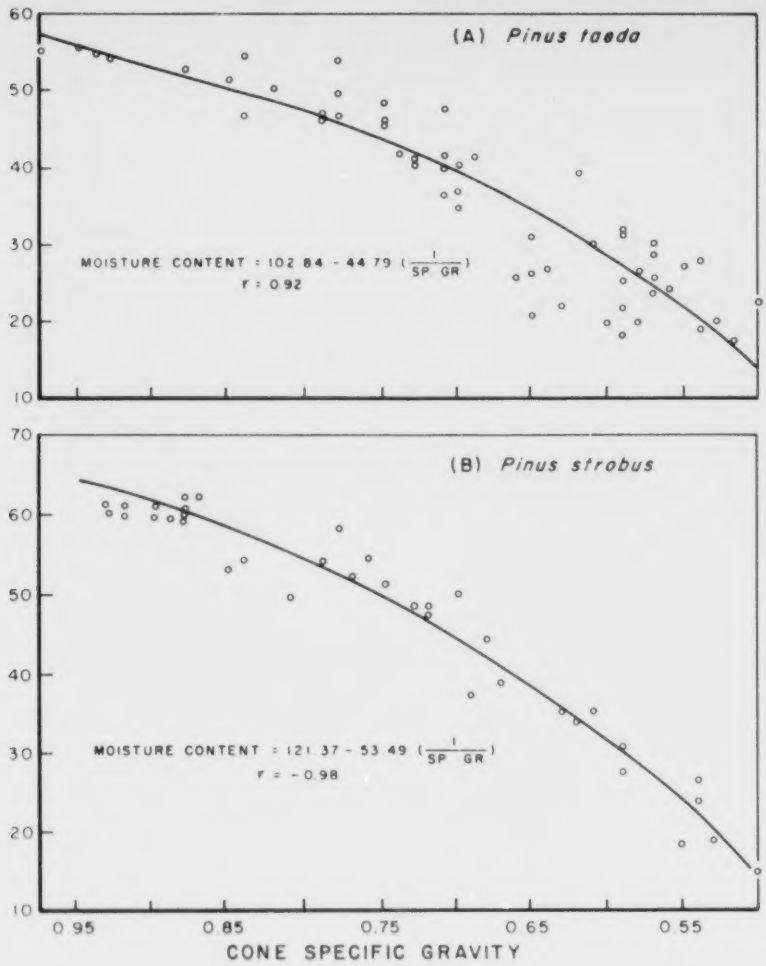

Figure 7.—*The relationship of moisture content to specific gravity for cones of* Pinus taeda *and* P. strobus *(Bonner 1991b).*

2. **Chemical characteristics**
   Chemical indices are biologically sound but not practical. They include:
   a. Accumulation of storage foods (fats and sugars)
   b. Elemental analyses of calcium, magnesium, and phosphorus for angiosperms of Southern United States
   c. Growth substances
      (1) Indoleacetic acid (IAA)
      (2) Gibberellins

H. Artificial Maturation
   Immature seeds can be artificially matured by picking the immature seeds, then ripening them under special storage conditions. However, seed yields and quality usually suffer. Artificial maturation includes the following considerations:
   1. Single-seed or multiple-seed fruits
   2. Avoiding dormancy through early collection
   3. Useful for collecting on remote or expensive sites

I. Delayed Collections
   For trees with serotinous (*Pinus* and *Picea*) or species with delayed fruit abscission (*Platanus* spp.), there is no rush to collect the fruits.

J. Sources
   For additional information, see Bonner 1972a, 1976; Nautiyal and Purohit 1985; Rediske 1961; Willan 1985, p. 33–38.

V. Postharvest Care

A. Introduction
   The time between collection and extraction is often overlooked as a crucial segment of seed acquisition. Fruits and seeds, often high in

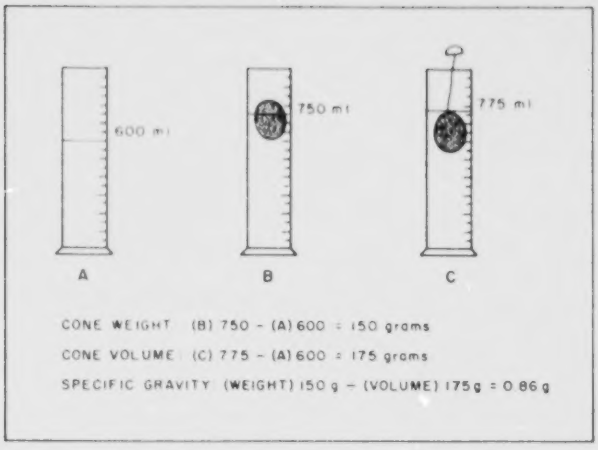
600 ml
750 ml
775 ml
A
B
C
CONE WEIGHT (B) 750 – (A) 600 = 150 grams
CONE VOLUME (C) 775 – (A) 600 = 175 grams
SPECIFIC GRAVITY (WEIGHT) 150 g – (VOLUME) 175 g = 0.86 g

Figure 8.—*A simple technique for determining specific gravity of pine cones in the field using a graduated cylinder. (A) Fill the cylinder with water to the 600 mL mark. (B) Float the cone in the water and record the water level. (C) Using a pin or needle, submerge the cone enough to completely cover the cone with water, but no more. Record the new water level (adapted from Barnett 1979).*

moisture content, must be stored and/or transported for extraction and cleaning. Special care must be taken during this period to avoid loss of seed quality, especially in tropical and subtropical areas where transportation systems do not allow immediate delivery to extraction centers.

B. Objectives
   1. Recognize the crucial times when seed quality may be lost.
   2. Plan storage and transportation systems to minimize the danger to seed quality.

C. Key Points

   The following points are essential to postharvest care:
   1. High moisture contents and high temperatures are dangerous for orthodox species.
   2. High moisture levels must be maintained in recalcitrant seeds, but excessive heat is a problem with these seeds.
   3. Fruit storage can be advantageous for some species because of the afterripening processes that occur in the seeds.

D. Storage Before Extraction
   1. **Operation schedules**—Time does not permit seeds from all trees or families to be collected at peak maturity; therefore, some must be picked and stored.
   2. **Predrying**—Drying during storage can remove enough moisture to lower drying costs.
   3. **Completion of maturation**
      a. 5 or 6 months in cool, moist conditions complete maturation for *Abies*.
      b. Similar benefits are possible for highest seed quality for some pines of the Southern United States.
      c. Premature collection is suitable for some multiseed hardwoods; e.g., *Liquidambar, Liriodendron,* and *Platanus*.

E. Southern Pines
   1. **Storage** is usually related to operation schedules.
   2. **Outdoor storage** is better than indoor storage.
   3. **Containers**
      a. All containers must provide air circulation among the cones.
      b. Burlap bags (about one-third hectoliter, loose weave) or wooden crates are best.
      c. Plastic bags or sacks should not be used.
      d. Paper sacks are satisfactory for small lots.
   4. **Time**
      a. Storage can improve germination rate.
      b. Maximum length of storage depends on the species.
   5. **Other factors**
      a. Original maturity of cones is important; more mature cones cannot be stored as long as less mature cones.
      b. Local weather is important; warm, rainy conditions increase the risk of cone molds.
   6. **Immaturity/Dormancy** can be changed during cone storage.
   7. **Heat and molds**
      a. Green cones can generate heat.
      b. External molds are common in some containers, but these molds may not cause damage.
      c. Good aeration is essential; it prevents mold growth on cones during drying.

F. Serotinous Cones
   1. Storage is not a major problem for *Pinus glauca, P. contorta,* or *P. patula*.
   2. Some pine seeds need to remain in the cones to reach maturity.

G. Other Conifers
   1. True firs (*Abies*) must complete ripening in the cones.
   2. Seeds of most *Picea* species should be extracted as early after collection as possible.
   3. *Pseudotsuga* cones can be stored for 3 to 4 months in dry, well-ventilated conditions.
   4. The recommendations for tropical pines are:

a. Cover with good ventilation, with temperatures between 20 and 35 °C.
b. Protect from rodents and fungi.
c. In Honduras, *Pinus caribaea* is precured until all of the cone changes from green to brown.
d. In New Zealand, immature cones of *P. radiata* are stored for 10 weeks at 20 to 24 °C.
e. In Indonesia, green and green/brown cones of *P. merkusii* are stored for 2 to 4 weeks.

H. Hardwoods
1. Immature fruits of some species will respond to artificial ripening, but seed yields and quality suffer.
2. Seeds of some species should be stored for as short a period as possible. Orthodox species include:
a. *Eucalyptus* – Store in tightly-woven cloth bags.
b. Legumes – Storage is easily managed.
c. Drupes – Short storage will help to complete ripening.

I. Summary
Most species fit into one of three groups:
1. **Harvest dry, keep dry** – Start drying immediately, and keep dry after extraction (e.g., *Pinus, Liquidambar, Liriodendron, Acacia,* and *Eucalyptus*).
a. Use a slow drying rate.
b. Provide good aeration.
c. Use suitable containers, including:
(1) Burlap bags
(2) Racks
(3) Wooden crates
(4) Canvas or plastic sheets
2. **Harvest moist, then dry** – Keep moist when collecting and during extraction, but dry seeds for storage (e.g., *Nyssa* and *Prunus*).
a. Spread to avoid heat.
b. Use trays or bags.
c. Avoid outer coat toughness.
d. Extract, wash, and dry for storage.
3. **Moist forever** – This method is used for recalcitrant seeds because drying decreases quality (e.g., *Quercus, Aesculus, Shorea,* and *Hopea*).
a. Never dry.
b. Keep moisture ≥30 percent.
c. Refrigerate to a safe temperature:
(1) 1 to 3 °C for temperate species
(2) 15 to 20 °C for tropical species
d. Use polyethylene-lined containers or bags.

J. Sources
For additional information, see Bonner 1987a; Willan 1985, p. 78–86.

Handling

I. Drying and Extracting

A. Introduction

Like agricultural seeds, many tree fruits dry as they mature, and seeds are extracted best at low moisture contents. Other tree seeds are still very moist at maturity, and special considerations are needed for extraction. No matter what type of fruit is involved, however, the objective of extraction is to obtain the maximum amount of seeds in the best physiological condition in an economically efficient operation. During extraction, seed quality can be greatly reduced by excessively heating the fruit to force opening or by extracting by hand or machine.

B. Objectives

1. Recognize potential problems of seed extraction related to the type of fruit.
2. Identify the basic techniques of tree seed drying and extraction.

C. Key Points

The following points are essential to seed drying and extracting:

1. For species that require drying, excessive heat in the presence of high moisture content can be deadly.
2. Seed damage can occur during mechanical separations.
3. Good training of workers is essential.
4. Extraction strategy depends on the type of fruit involved.

D. Multiseed Fruits

Multiseed fruits include pods; moist, fleshy fruits; and cones and capsules. Each type requires different steps to extract the seeds:

1. **Pods**
   a. Dry the fruits.
   b. Thresh manually by:
      (1) Flailing with poles
      (2) Crushing by trampling
      (3) Hitting with heavy mallets
   c. Thresh mechanically by:
      (1) Slow, rotating drums (cement mixer)
      (2) CSIRO flailing thresher
      (3) Dybvig macerator
      (4) Hammer mills or other flailing devices
   d. Use a series of steps for difficult species.
2. **Moist, fleshy fruits**
   a. Start quickly to avoid fermentation.
   b. Soak in water.
   c. Extract with macerators, mixers, coffee depulpers (*Gmelina arborea*), feed grinders, hammer mills, etc.
   d. Run small fruits (e.g., *Rubus* and *Morus*) in blenders at slow speeds with lots of water.
   e. Use water extraction with a high-pressure stream.
3. **Cones, capsules, and other multiple fruits**
   a. Air-dry on flat surfaces.
      (1) Canvas is best for large quantities.
      (2) Screen trays are good for smaller lots, such as single-tree collections.
      (3) Plastic sheets are not strong enough for *Pinus* cones (1 hL of cones equals 35 kg).
      (4) Protect the drying fruits from rain and predators; spread thin and stir frequently.
      (5) Dry some species under shade (e.g., *Hopea* spp., *Triplochiton schelroxylon*, and *Pinus oocarpa*).
   b. Use solar kilns.
      (1) Simple type (clear polyethylene stretched over a frame)
      (2) Solar heat storage units (more sophisticated)
   c. Use heated kilns for large quantities of cones; some types are:
      (1) Progressive (cone containers are moved along a gradient of increasing temperature)
      (2) Large batch (large, heated chambers in which trays hold cones and rotate positions)
      (3) Small batch (wire-bottom drawers that hold about one-third hL of cones each)
      (4) Stack trays (wooden trays with perforated sheet-metal bottoms, not wire screens, in stacks of six with eight stacks heated with one heating system)
      (5) Tumbler driers (cylindrical batch kilns that rotate while drying)
      (6) Other batch kilns (many local designs available)
   d. Set temperature and humidity parameters. The object is to remove moisture; high temperatures create a greater vapor pressure gradient. Some recommended parameters are 29 to 50 °C for conifers, and 8 hours at 60 °C for *Eucalyptus saligna*. Use a 15-second dip in boiling water for serotinous cones to melt the resin before placing them in the kiln.
   e. Extract the seeds after the cones are open with:
      (1) Tumbler driers
      (2) Cement mixers
      (3) Homemade tumblers

E. Single-Seed Fruits
Single-seed fruits include drupes (e.g., *Prunus* and *Vitis*) and nuts.
1. For drupes or other fleshy fruits, use macerators, mixers, etc.
2. For nuts with husks, use macerators or hand rubbing.

F. Sources
For additional information, see Willan 1985, p. 87–111.

II. Cleaning and Upgrading

A. Introduction
Cleaning seedlots is a basic step in proper seed utilization. Cleaning should remove wings or other seed appendages, empty seeds, damaged seeds, and nonseed trash. This cleaning should also provide dramatic decreases in insect and disease problems. Many seedlots can be upgraded by removing immature, damaged, and dead seeds after the initial cleaning. Many people view large mechanical operations as the only way to clean and upgrade seedlots, but seedlot quality can be improved with simple equipment and techniques.

B. Objectives
1. Learn the advantages of cleaned and upgraded seedlots.
2. Become familiar with the principles of seed-cleaning equipment and techniques.
3. Apply these principles when seed cleaning and upgrading are planned.

C. Key Points
The following points are essential for seed cleaning and upgrading:
1. Liquid flotation can be an essential aid for many species, especially recalcitrant ones.
2. Screen cleaning is the basic seed-cleaning method.
3. Air separation, including winnowing, is a valuable technique.
4. Cleaning small lots for testing or research may be very different from cleaning large lots.
5. Upgrading seedlots offers potential improvements in eight areas.
6. Seed sizing can be useful for some species or sources but not for others.

D. Cleaning
1. **Flotation**—The simplest method of all.
   a. Initial moisture content is crucial.
   b. Orthodox seeds are redried after flotation, but recalcitrant seeds are not.
   c. Flotation
      (1) Removes light trash.
      (2) Removes many empty, broken, diseased, or insect-damaged seeds.
      (3) Is very good for large seeds with high moisture contents.
2. **Aspirators**—Any machine that uses air to clean and separate:
   a. Large-scale machines in seed plants
   b. Small-lot cleaners for testing laboratories and research
      Some types are:
      (1) General ER
      (2) South Dakota
      (3) Stults
      (4) Barnes
      (5) Other models as described by Willan 1985
      (6) Homemade fan devices
      (7) Carter Day Duo Aspirator
3. **Screens and sieves**—There are two types of screening devices:
   a. Hand screens
   b. Mechanical screen cleaners
4. **Air-screen cleaners**—These are the basic seed-cleaning machines in most seed plants. They combine aspiration and screening. Even small models can clean 30 to 40 kg of small seeds per hour. Important principles to remember are:
   a. They perform three functions:
      (1) Scalping
      (2) Sizing
      (3) Aspiration
   b. They are more efficient as cleaners, not as sizers.
5. **Electrostatic cleaners**—The Helmuth machine is good for very small seeds.
6. **Dewinging**—A special type of cleaning that reduces storage volume, makes upgrading possible, makes sowing easier, and removes pathogens. There are two basic methods of dewinging—wet and dry.
   a. The dry method is recommended for tough seeds only because of the damage potential to thin seed coats. Dry dewingers include:
      (1) Popcorn polishers
      (2) Missoula Equipment Development Center dewinger
      (3) Dybvig
      (4) Electric drum
      (5) New conifer dry dewingers
   b. The wet method is usually preferred for conifers. Wet dewingers include:
      (1) Cement mixers
      (2) Commercial dewingers
      (3) Kitchen blenders (for small lots)
      (4) Any cylinder with gentle agitation

E. Upgrading

1. **Upgrading** is improving the potential performance of a seedlot by removing empty, damaged, weak, immature, or odd-sized seeds.
2. **Upgrading will:**
   a. Remove weak seeds
   b. Remove empty seeds
   c. Reduce chances of insect and disease damage
   d. Improve control of density in nursery
   e. Reduce planting time
   f. Facilitate nursery operations
   g. Reduce costs and improve uniformity
   h. Reduce storage space requirements
3. **Methods and equipment**
   a. Specific gravity by flotation uses:
      (1) Water for some *Pinus*, *Quercus*, and other large seeds
      (2) Organic solvents (usually alcohols) of different densities for some small seeds
   b. Air-screen cleaners:
      (1) Separate by three physical properties
      (2) Upgrade by sizing or by removing empties with air
      (3) Regulate screen pattern, feed rate, airflow, screen oscillation (pulleys), and screen pitch (in some models)
   c. Air separators include:
      (1) Large air-column separators
      (2) Fractionating aspirators
      (3) Small laboratory blowers
   d. Gravity separators were originally built to remove ore from clay. They:
      (1) Can separate seeds of the same size and different densities or different sizes and the same density.
      (2) Are widely used on conifer seeds in North America.
      (3) Regulate feed rate, air stream through deck, deck pitch (side and end), and eccentric thrust.
   e. Electrostatic separators create a charge that adheres to the seed surfaces. Models include:
      (1) Helmuth cleaner for *Eucalyptus* and conifers of the Western United States.
      (2) Static electricity for very small seeds. The sides of a plastic beaker are wiped with a nylon cloth.
   f. Radiography is used for valuable research lots only.
   g. Color separators remove light-colored seeds.
   h. Incubation, drying, and separation (IDS) method—A new method from Sweden that is used on *Pinus* and *Picea*.
4. **Sizing** helps with some species or seedlots, but not with others; e.g., single family seedlots.

G. Sources

For additional information, see Bonner 1987b; Doran and others 1983, chap. 5; Willan 1985, p. 87–128.

III. Storage Principles

A. Introduction

The primary purpose of storing seeds is to have a viable seed supply when it is needed for regeneration. Successful storage of woody plant seeds must be carefully planned, and good planning depends on an understanding of the purposes of storage, of seed deterioration, and of the effects of the storage environment on the deterioration processes.

B. Objectives

1. Learn the objectives and rationale of seed storage.
2. Identify factors that affect seed longevity in storage.
3. Review the general process of seed deterioration.

C. Key Points

The following points are essential to understanding seed storage principles:

1. Longevity of seeds is a species characteristic.
2. Prestorage factors affect longevity in storage.
3. The most important factors in storage are seed moisture content and temperature.
4. Seed deterioration begins at abscission and involves complex physiological changes.

D. Objective of Storage

The objective of storage is to delay deterioration or decrease its rate until seeds are used.

E. Rationale for Storage

Storage may be short- or long-term; it may be extended for long periods for germplasm conservation.

1. **Short-term storage:**
   a. Is used for immediate operations
   b. Typically lasts less than 5 years
   c. Allows carry-over of surplus production
   d. Requires minimum storage space requirements
2. **Long-term storage:**
   a. Typically lasts from 5 to 10 years
   b. Ensures constant seed supply

c. Saves special lots that will not be collected annually
d. Requires very good storage environments

3. **Germplasm conservation**
   a. If storage is planned for 50 years or more
   b. Requires the very best storage environment

F. Longevity in Storage

Many factors affect seed longevity in storage:

1. **Seed characteristics**
   a. Basic physiology
      (1) Orthodox seeds are tolerant of desiccation to low moisture contents.
      (2) Recalcitrant seeds are intolerant of desiccation.
   b. Seed structure – Thick or hard seedcoats restrict moisture uptake and gas exchange.
   c. Seed chemistry – Oily seeds tend to be harder to store than starchy seeds.
   d. Stage of maturity – Immature seeds usually will not store as well as fully mature seeds.
   e. Environmental stress – Stress during maturation can affect longevity.
2. **Seed handling before storage**
   a. Physiological mistreatment can damage storage potential.
   b. Processing damage will lower seed quality.
3. **Genetics** – Good seed quality is inherited to some degree.
4. **Storage environment**
   a. Moisture content
      (1) Moisture content is the most important factor.
      (2) Potential damage thresholds are outlined in table 5.
      (3) The best range for orthodox seeds is 5 to 10 percent.
      (4) The best range for recalcitrant seeds is full imbibition.

Table 5. – *Moisture content thresholds and potential effects on stored seeds*

Moisture content	Effects
Percent	
>30	Germination begins
18 to 20	Overheating from respiration
10 to 18	Seed fungi become active
>9	Insect activity
5 to 8	Best range for sealed storage
<5	Desiccation damage possible in some species

Table 6. – *Equilibrium moisture contents at 4 to 5 °C and three relative humidities (Bonner 1981b, Justice and Bass 1978)*

	Relative humidity		
	Percent		
Species	20	45	95
	Moisture content		
	Percent		
Orthodox trees			
Carya ovata	...*	10	15
Juglans nigra	...	11	20
Liquidambar styraciflua	...	8	20
Liriodendron tulipifera	...	10	19
Picea abies	6	8	...
Pinus sylvestris	6	8	...
P. taeda	...	10	17
Prunus serotina	...	9	17
Orthodox crops			
Glycine max	6	8	19
Zea mays	8	12	20
Recalcitrant trees			
Quercus alba	...	37	50
Q. nigra	...	13	29
Shorea robusta	...	...	35

*Data not available.

(5) The equilibrium moisture content is defined as the seed moisture content when seed moisture is in equilibrium with the moisture in the storage atmosphere (table 6). Equilibrium moisture content:
   (a) is influenced by seed chemistry (fig. 9)
   (b) is rarely reached with recalcitrant seeds
   (c) has sorption and desorption differences

b. Temperature
   (1) Generally, the cooler the seeds, the slower the deterioration rate.
   (2) The safe temperature range for orthodox seeds is related to the moisture content of the seeds:
      (a) Orthodox seeds at 5- to 10-percent moisture can be stored at most temperatures.
      (b) Between 50 and 0 °C, every 5 °C lowering of storage temperature doubles the life of the seeds (Harrington 1972).
   (3) The safe temperature ranges for recalcitrant seeds are:
      (a) Temperate Zone species: −1 to 3 °C.
      (b) Tropical species: usually above 12 to 15 °C.

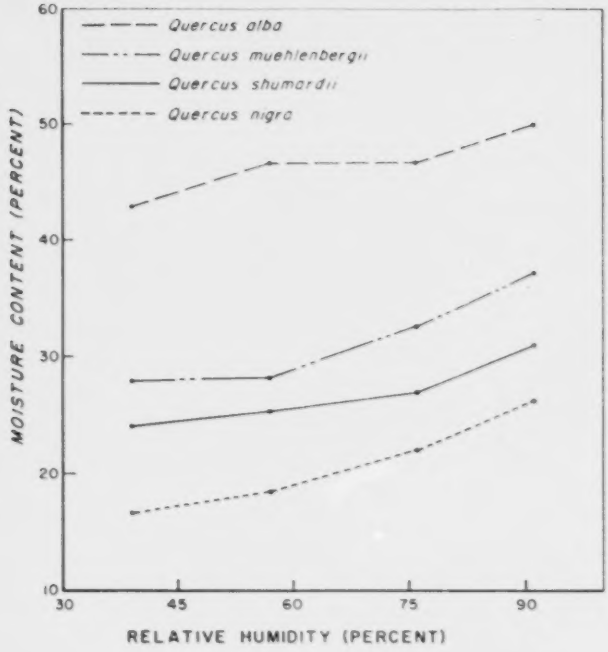

Figure 9.—*Equilibrium moisture content at 25 °C for four recalcitrant* Quercus *species (adapted from Willan 1985).*

c. Storage atmosphere
   (1) If oxygen levels are reduced, metabolism is slowed, which can increase longevity.
   (2) Inert gases offer no advantage in long-term storage, but they may help in short-term storage.
   (3) In sealed containers, the CO_2/O_2 ratio changes.

G. Cells and Tissues During Seed Aging
The following changes occur in cells and tissues during aging:
1. Loss of food reserves
2. Accumulation of metabolic byproducts
3. Irreversible enzyme deactivation
4. Deterioration of cell membranes
5. Lipid peroxidation
6. Alterations of DNA

H. Sources
For additional information, see Bonner and Vozzo 1990; Harrington 1972; Justice and Bass 1978; Tang and Tamari 1973; Willan 1985, p. 129–160.

IV. Storage Applications

A. Introduction
The previous section introduced the principles and critical factors that influence seed longevity. This section discusses how these principles are applied in practice to store tree seeds.

B. Objectives
1. Relate seed storage principles to prescriptions for each species group.
2. Learn features of cold storage units.
3. Discuss storage constants and their application.
4. Learn basic principles of seed management in storage.

C. Key Points
The following points are essential to storage applications:
1. There are four classes of seed storage behavior.
2. Cold storage is best but not always necessary for successful seed storage.
3. Each species, and perhaps individual populations within a species, will nearly always respond identically to a given type of storage conditions.
4. Good facilities and good seeds are not enough; good management is essential for optimum seed storage operations.

D. Seed Storage Classes
There are four classes of tree seed storage behavior (table 7):
1. **True orthodox**
   a. True orthodox seeds are tolerant of desiccation (table 8) and:
      (1) Can be dried to moisture levels of 5 to 10 percent.
      (2) Can be stored at subfreezing temperatures.
      (3) Are easily stored for at least one rotation.
      (4) Have generally unknown upper limits of storage.
   b. Examples include most of the valuable temperate genera (*Pinus, Picea, Betula, Prunus)* and many tropical genera (*Acacia, Eucalyptus,* and *Casuarina*).
2. **Suborthodox**
   a. Suborthodox seeds are similar to true orthodox seeds but are limited to shorter periods (table 9).
      (1) They are stored under the same conditions as true orthodox seeds.
      (2) They are limited in storage potential because of high lipid contents, thin seedcoats, or genetic makeup.
   b. Examples include those with high lipid content (*Juglans, Carya,* some *Abies,* and *Pinus*), those with thin seedcoats (*Populus* and *Salix*), and some whose genetic makeup requires slow drying (*Fagus* and *Citrus*).

Table 7.—*Storage conditions for four storage classes of tree seeds*

Storage class	Storage period	Seed moisture	Temperature	Container type
	Years	*Percent*	°C	
True orthodox	<5	6–10	0–5	Airtight
	>5	6–10	−18	Airtight
Suborthodox	<5	6–10	0–5	Airtight
	>5	6–10	−18	Airtight
Temperate recalcitrant	<3	30–45	−1 to −3	4-mil* plastic, unsealed
Tropical recalcitrant	<1	30–45	12–20	4-mil plastic, unsealed

*mil = 1/1,000 inch = 0.025 mm.

Table 8.—*Storage test results for true orthodox species (adapted from Bonner 1990)*

Species	Storage conditions		Storage results	
	Temperature	Seed moisture	Time stored	Viability loss
	°C	*Percent*	*Years*	*Percent*
Abies procera	0	9	7.0	11
Acacia leptopetala	20–25	...*	18.0	1
A. mangium	4–8	...	1.2	6
A. pruinocarpa	20–25	...	16.0	20
Acer saccharum	−10	10	5.5	5
Albizia falcataria	4–8	...	1.5	10
Araucaria cunninghamii	−15	16–23	8.0	few†
A. cunninghamii	19	7	0.1	0
Casuarina equisetifolia	−3	6–16	2.0	0–5
C. torulosa	20–25	8–12	18.0	6
Liquidambar styraciflua	3	5–10	9.0	3
Pinus caribaea var. *hondurensis*	8	...	2.7	±16
P. elliottii	4	10	50.0	30
P. merkusii	4–5	<8	4.0	0
P. ponderosa	0	8	7.0	0
Tectona grandis	0–4	≈12	7.0	0
Tsuga heterophylla	5	8	2.0	0
T. heterophylla	−18	8	2.0	0

*Data not available.
†Exact value not available from original source.

Table 9.—*Storage test results for suborthodox species (adapted from Bonner 1990)*

Species	Storage conditions		Storage results	
	Temperature	Seed moisture	Time stored	Viability loss
	°C	*Percent*	*Years*	*Percent*
Citrus limon	−20	5	0.9	±5
Fagus sylvatica	−10	10	5.0	34
Gmelina arborea	−5	6–10	2.0	10
Populus deltoides	−20	6–10	6.0	21
Salix glauca	−10	6–10	1.2	0

3. **Temperate recalcitrant**
   a. Temperate recalcitrant seeds are intolerant of desiccation (table 10)
      (1) They cannot be dried below 20- to 30-percent moisture; therefore, storage must be above freezing.
      (2) They have metabolisms so rapid that pregermination commonly occurs in storage.
      (3) They cannot be stored in airtight containers; there must be some gas exchange (table 7).
   b. Examples include *Quercus* and *Aesculus*.
4. **Tropical recalcitrant**
   a. Tropical recalcitrant seeds are the same as temperate recalcitrant seeds but are sensitive to low storage temperatures (table 11). They experience chilling damage and death below 12 to 20 °C.
   b. They are the most difficult group of all to store.
   c. Examples include *Shorea, Hopea,* and *Dipterocarpus,* and even some legumes (*Pithocellobium* spp. in Costa Rica).

Table 10.—*Storage test results for temperate recalcitrant species (adapted from Bonner 1990)*

Species	Storage conditions		Storage results	
	Temperature	Seed moisture	Time stored	Viability loss
	°C	*Percent*	*Months*	*Percent*
Acer saccharinum	−3	50	18	8
Quercus falcata var. *pagodaefolia*	3	35	30	6
Q. robur	−1	40–45	29	31–61
Q. rubra	−1 to −3	38–45	17	18–46
Q. virginiana	2	...*	12	35

*Data not available.

Table 11.—*Storage test results for tropical recalcitrant species (adapted from Bonner 1990)*

Species	Storage conditions		Storage results	
	Temperature	Seed moisture	Time stored	Viability loss
	°C	*Percent*	*Days*	*Percent*
Araucaria hunsteinii	19.0	25–30	54	±30
A. hunsteinii	2.0	30	365	82
Azadirachta indica	26.0	10–18	56	65
Hopea helferi	15.0	47	37	2
Shorea robusta	13.5	40–50	30	60
S. roxburghii	16.0	40	270	±30

E. Cryogenic Storage
Cryogenic storage is a method for very long-term storage for germplasm conservation (table 12).
   1. **Techniques**
      a. Packages are immersed in liquid nitrogen (−196 °C) or suspended above it in the vapor.
      b. It has potential for small quantities only.
      c. Maximum time limits are not known. Only a few tests have been made on tree seeds.
   2. **Costs**—Costs are comparable with conventional storage in some cases.

F. Physical Facilities
   1. **Cold storage units**
      a. Cold storage units require a reliable power source, should not be built where floods or earthquakes are likely, should be located near other seed activities, should be rodent proof, and should be on high elevations when possible because ambient temperatures will be cooler.
      b. Units should be built to hold a 5-year supply.
      c. For germplasm conservation, about 1 liter is needed of each sample; e.g., 85 m^3 should hold 22,800 samples.
      d. Humidity control is not recommended in the Tropics.
      e. Direct or indirect vapor-compression refrigeration is recommended.
      f. Standby generators are needed.
      g. Thermal time constants of 4 to 5 days should apply in large coolers.
      h. Modular panel units are effective.
      i. Insulation depends on ambient conditions.

Table 12.—*Storage test results for cryogenic trials of forest tree seeds (adapted from Bonner 1990)*

Species	Seed moisture	Time stored	Viability loss
	Percent	*Days*	*Percent*
Abies alba	...*	6	5
A. concolor	<13	180	0
Fagus sylvatica	...	6	100
Larix decidua	...	6	5
Picea abies	...	6	1
Pinus echinata	...	112	0
P. ponderosa	<13	180	0
P. sylvestris	...	6	0
Populus tremula × *tremuloides*	...	6	1
Ulmus pumila	...	112	0

*Data not available.

2. **Containers**
   a. Fiber drums
   b. Rigid plastic containers, which are better than glass
   c. Rectangular containers
   d. Plastic bags
3. **Moisture management**
   a. Seeds will reach an equilibrium moisture content when exposed to the storage atmosphere.
   b. With humidity control (50 to 60 percent relative humidity), orthodox seeds need not be sealed. Recalcitrant seeds cannot be sealed, so they cannot be stored in such a unit.
   c. Without humidity control (>95 percent relative humidity), recalcitrant seeds store well. Orthodox seeds must be dried and stored in sealed containers.
   d. Humidity controls are not recommended for the Tropics.
   e. Frost-free refrigerators are an inexpensive alternative for small quantities.

G. Genetic Damage in Long-term Storage
   1. Would be devastating to seeds stored for germplasm conservation, but there is no strong evidence to date of lasting damage.
   2. Could cause changes in the population.

H. Retesting in Long-term Storage
   For retesting in long-term storage:
   1. Use ISTA rules or comparable procedures.
   2. Use the following test interval for orthodox seeds: initial year, third year, and every fifth year thereafter.
   3. Ensure nondestructive testing.
   4. Regenerate when viability falls to 50 percent.

I. Viability Constants in Storage
   1. **Theory**—Viability retention will be the same for a given species under a given set of storage conditions.
   2. **Practice**
      a. Results are good with some agricultural seeds.
      b. Varieties of a single species may differ.
      c. One must start with very good seeds.
      d. There are few data for tree seeds.
   3. **Viability constants**—If valid, they could be very useful in planning long-term storage for germplasm conservation.

J. Sources
   For additional information, see Bonner and Vozzo 1990; Chin and Roberts 1980; Harrington 1972; International Board for Plant Genetic Resources 1976; Justice and Bass 1978; Roberts 1973; Tang and Tamari 1973; Willan 1985, p. 129–160.

Evaluating Quality

I. Sampling

A. Introduction

Sampling is the process of taking a small part or quantity of something for testing or analysis; it is the first step in seed testing. In sampling, it is essential to obtain: (1) a sample of proper size and (2) a sample representative of the main seedlot. The results of the laboratory tests can only show the quality and characteristics of the sample submitted for the analysis; therefore, the validity of test results for a large seedlot is determined by the success of obtaining a representative sample. Sampling seedlots for quality evaluation must be done systematically, using appropriate techniques, tools, and procedures, to ensure that the seed sample represents the entire lot.

B. Objectives

1. Quantify a seedlot according to accepted standards.
2. Determine sampling intensity according to size and characteristics of the seedlot.
3. Learn appropriate sampling instruments and techniques according to recognized standards.

C. Key Points

The following points are essential in seed sampling:

1. Laboratories can only measure the properties of the sample; the sampler must ensure that the sample truly represents the seedlot.
2. Submitted samples should contain at least 2,500 seeds (except for very large seeds of certain species).
3. Drawing the sample must be completely random.
4. Proper packaging and labeling of the sample are essential.

D. Definition of Terms

Relevant terms are defined as follows:

1. **Lot** – a specified, physically identifiable quantity of seeds.
2. **Primary sample** – a small quantity of seeds taken from one point in a seedlot
3. **Composite sample** – formed by combining and mixing all the primary samples taken from a seedlot
4. **Submitted sample** – the sample submitted to the testing laboratory
5. **Working sample** – a subsample taken from the submitted sample in the laboratory
6. **Subsample** – a portion of a sample obtained by reducing the sample by recognized methods (table 13).

Table 13. – *Weights of lots and samples for shrubs and trees (ISTA 1985)*

Species	Maximum weight of seedlot	Submitted sample	Working sample for purity analysis
	Kilograms	*Grams*	*Grams*
Acacia spp.	1,000	70	35
Ailanthus altissima	1,000	160	80
Alnus rubra	1,000	15	2
Castanea sativa	5,000	500 seeds	500 seeds
Cedrela spp.	1,000	80	40
Eucalyptus camaldulensis	1,000	15	5
E. globulus	1,000	60	20
E. tereticornis	1,000	15	5
Morus spp.	1,000	20	5
Pinus halepensis	1,000	100	50
P. wallichiana	1,000	250	125
Quercus spp.	5,000	500 seeds	500 seeds
Robinia pseudoacacia	1,000	100	50

E. Sampling Intensity

A sample is obtained by selecting small portions at random from various positions in a seedlot and combining them.

1. **Calculating primary samples** – Each composite sample must be made up of at least five primary samples.
2. **Seedlot size** – For international trade in tree seeds, a maximum size of a seedlot for most species has been set at 1,000 kg ±5 percent (table 13).

F. Sampling Procedures

There are three common sampling tools or techniques:

1. **Triers** are used for free-flowing seeds. The steps are:
   a. Close the gates before inserting the trier into the drum.
   b. Insert the trier into the drum.
   c. Open the gates.
   d. Close the gates.
   e. Remove the trier.
   f. Dump the seeds.
2. **Soil dividers** are used primarily for small lots. The steps are:
   a. Pour the seeds through the divider several times for mixing.
   b. Divide the sample into halves, quarters, etc.
3. **Extended hand method** is used for chaffy, winged, or other nonflowing seeds. The steps are:
   a. Extend the fingers, and insert the hand straight into the seeds.
   b. Close the hand, and withdraw a primary sample.

G. Preparation of the Sample
   1. **Composite sample** – All primary samples are combined and mixed (table 13).
   2. **Working sample** – The submitted sample is reduced to a working sample by:
      a. Mechanical divider method
      b. Random cups method
      c. Modified halving method
      d. Spoon method
      e. Manual halving method
   3. **Extra seeds** – The remainder of the submitted sample should be stored to permit retesting if necessary. International Seed Testing Association (1985) recommends storing for 1 year.

H. Sources
   For additional information, see Association of Official Seed Analysts 1988, Edwards 1987, International Seed Testing Association 1985.

II. Moisture Content

A. Introduction
   The first measurements taken in seed testing are moisture, purity, and weight. All of these measurements are important, but moisture is the most critical one. Seed moisture levels can influence or indicate seed maturity, longevity in storage, and the amount of pretreatment needed for rapid germination.

B. Objectives
   1. Learn the principles of official seed testing for moisture.
   2. Apply these principles in practical exercises.

C. Key Points
   The following points are essential to testing for moisture content:
   1. Official testing procedures are prescribed in detail.
   2. Many tests may be unofficial, and different methods may be used, but accuracy and precision are still essential.
   3. Large recalcitrant seeds present special problems that official testing rules have not yet adequately addressed.

D. Definition of Terms
   Relevant terms are defined as follows:
   1. **Sample, submitted** – the sample of seeds submitted to a seed-testing station; it should be twice the size of the working sample.
   2. **Sample, working** – a reduced seed sample taken from the submitted sample in the laboratory
   3. **Seedlot** – a specified quantity of seeds of reasonably uniform quality

E. Moisture Measurements
   1. **Importance**
      a. Is the most important factor in viability retention.
      b. Controls insect and disease activity (table 5).
      c. Affects the relationship of weight to number of seeds.
   2. **Frequency** – Moisture is measured:
      a. After extracting and cleaning.
      b. When seeds are placed in storage.
      c. Periodically during storage.
      d. When seedlots are shipped.
   3. **Procedures** – Accurate results are ensured by:
      a. Using the submitted sample.
      b. Measuring immediately on receipt.
      c. Expressing results as a percentage of fresh weight (wet weight), not dry weight.
   4. **Methods** – Moisture content can be measured by four methods:
      a. Ovendrying method – Critical points are:
         (1) Heat samples for 17 ±1 hours at 103 ±2 °C.
         (2) Use forced-draft ovens.
         (3) Place samples in glass or metal containers.
         (4) Leave space between cans in the oven.
         (5) Cool the samples in desiccators.
         (6) Keep ambient humidity less than 70 percent in the laboratory if possible.
         (7) Weigh to the nearest milligram.
         (8) Grind or cut large seeds or seeds of high moisture content.
         (9) Predry if seed moisture exceeds 17 percent in seeds that must be ground, or 30 percent in other species.
         (10) Check tolerance on results. Moisture tolerances for tree seeds are more liberal than those for agricultural seeds (table 14).

Table 14. – *Tolerance levels for differences between two determinations of moisture content of tree and shrub seeds (ISTA 1985)*

Seed size class	Seeds per kilogram	Initial moisture	Tolerance
	Number	---------- *Percent* ----------	
Small seeds	>5,000	<12	0.3
Small seeds	>5,000	>12	0.5
Large seeds	<5,000	<12	0.4
Large seeds	<5,000	12–25	0.8
Large seeds	<5,000	>25	2.5

b. Electric meters:
   (1) Are not allowed for official ISTA tests but are very useful.
   (2) Are based on electrical resistance or capacitance and are accurate to within ± 1 percent on free-flowing seeds.
   (3) Require construction of calibration charts.
   (4) Are available in various models:
      (a) Motomco – based on capacitance and very accurate
      (b) Radson (Dole or Seedburo) – a reliable model in the United States
      (c) Dickey-John or Insto – based on capacitance
      (d) Super-Beha – widely used in Europe
c. Infrared instruments are small, infrared ovens with built-in balances, which use a gravimetric method based on drying time.
d. Laboratory methods for research:
   (1) Karl Fischer method
   (2) Toluene distillation
   (3) Nuclear magnetic resonance (nondestructive)
   (4) Infrared spectroscopy

F. Summary – See table 15.

G. Sources
For additional information, see Bonner 1981b; International Seed Testing Association 1985, sections 9, 9A; Willan 1985, p. 227–230.

III. Purity and Weight

A. Introduction
After moisture content has been determined, the submitted sample is ready for purity and weight determinations. These determinations are a vital part of official seed testing and practical seed use, with legal ramifications in both domestic and international seed trade.

Table 15. – *Suggested test procedures for tree seed moisture (Bonner 1981b)*

Seed size class	Accurate measurement or ISTA official test	Rapid estimate
Small seeds, low oil content (e.g., *Platanus, Robinia*)	Oven: 103 ± 2 °C for 17 ± 1 hours. Sample: 4 to 5 g	Electric meter Sample: 80 to 200 g, depending on type
Small seeds, high oil content (e.g., *Abies, Pinus, Tsuga, Zanthoxylum*)	Oven: 103 ± 2 °C for 17 ± 1 hours. Sample: 4 to 5 g or Toluene distillation	Electric meter Sample: 80 to 200 g, depending on type
Large seeds, low oil content, moisture <20% (e.g., *Nyssa*)	(1) Grind or equivalent (2) Oven: 103 ± 2 °C for 17 ± 1 hours. Sample: 4 to 5 g or enough to equal weight of five seeds	Microwave drying Sample: 4 to 5 g or enough to equal weight of five seeds
Large seeds, low oil content, moisture >20%, (e.g., *Aesculus, Quercus*)	(1) Predry to <20% at 130 °C for 5 to 10 minutes (2) Grind or equivalent (3) Oven: 103 ± 2 °C for 17 ± 1 hours. Sample: enough to equal weight of five seeds	Microwave drying Sample: enough to equal weight of five seeds
Large seeds, high oil content (e.g., *Carya, Fagus, Juglans*)	(1) Grind or equivalent (2) Oven: 103 ± 2 °C for 17 ± 1 hours. Sample: enough to equal weight of five seeds or Toluene distillation	Microwave drying Sample: enough to equal weight of five seeds

B. Objectives
   1. Learn the principles of official seed testing for purity and weight.
   2. Apply these principles in practical exercises.

C. Key Points
   The following points are essential to determine seed purity and weight:
   1. The line between true seeds and trash can be ambiguous for some tree seeds, especially those that are dewinged.
   2. Patience and good eyesight are needed.
   3. The smaller the seeds, the more difficult the purity test will be.

D. Definition of Terms
   Relevant terms are defined as follows:
   1. **Purity**—proportion of clean, intact seeds of the designated species in a seedlot, usually expressed as a percentage by weight
   2. **Sample, submitted**—the sample of seeds submitted to a seed-testing station; it should be twice the size of the working sample
   3. **Sample, working**—a reduced seed sample taken from the submitted sample in the laboratory on which some test of seed quality is made
   4. **Seedlot**—a specified quantity of seeds of reasonably uniform quality; maximum lot size is 1,000 kg (5,000 kg for *Fagus* and larger seeds)

E. Purity
   1. **Procedure**—The ISTA (1985) rules are followed for purity testing. The steps are:
      a. Reduce the submitted sample (after mixing) to the working sample by:
         (1) Mechanical dividers
         (2) Random cups
         (3) Modified halving
         (4) Spoon method
         (5) Manual halving (chaffy, winged, and large seeds)
      b. Divide the working sample into fractions of
         (1) Pure seeds
         (2) Other seeds
         (3) Inert matter
      c. Weigh and express each as a percentage of the total sample weight
   2. **Pure seed component**—This component contains:
      a. Intact seed units of the desired species
      b. Pieces of seed units larger than one-half the original size, even if they are broken
   3. **Tree seed specifics**
      a. Seeds of Leguminosae, Cupressaceae, Pinaceae, and Taxodiaceae with seedcoats entirely removed are inert matter.
      b. In *Abies, Larix, Libocedrus, Pinus elliottii, P. echinata, P. rigida, P. taeda,* and *Pseudotsuga,* wings or wing fragments are detached and removed and placed in the inert matter fraction. Other *Pinus* spp. retain wing fragments (see "a" above).
      c. For samaras, wings are not removed (e.g., *Acer, Fraxinus, Cedrela,* and *Swietenia*).
      d. For drupes, the fleshy coverings are not removed.
      e. In *Eucalyptus,* for species with small seeds, a simplified procedure is used; only other seeds and inert matter that is obviously of nonseed origin are removed.
      f. For Leguminosae, if any portion of the testa is present, it must be classified as pure seed.
      g. If species distinctions are impossible, only the genus name is given on the certificate.

F. Seed Weight
   1. **Determination**—The ISTA (1985) rules are used to properly determine seed weight. Either the whole working sample or replicates from it are used.
      a. Working sample—Weigh the entire pure seed fraction.
      b. Replicates—Count and weigh 8 replicates of 100.
   2. **Reporting results**—Results are reported in one of two ways:
      a. 1,000-seed weight
      b. Seeds per gram (or per kilogram, ounce, or pound)

G. Sources
   For additional information, see International Seed Testing Association 1985, sect. 3, 3A, 10; Willan 1985, p. 198–202, 221.

IV. Germination Tests

A. Introduction
   Good seed testing is the cornerstone of any seed program, no matter what kind of seeds: agricultural, forestry, agroforestry, or ornamental. The quality of the seeds used must be measured and described. Seed testing may have legal ramifications because of its connection to seed sales. For this reason, the International Seed Testing Association (ISTA) coordinates international efforts to standardize seed testing. The quality of seeds must be known to make efficient and effective use of them in reforestation or afforestation programs.

B. Objectives

1. Identify the international organizations that deal in tree seed testing and how they derive their prescriptions.
2. Learn the principles of germination testing and how they are applied in the laboratory for standard conditions.
3. Practice actual germination testing in the laboratory.
4. Learn proven techniques to analyze germination data and how these data can be expressed.
5. Learn the application of germination test results to practical nursery and field conditions.
6. Learn techniques for rapid estimates of seed quality when time and/or proper facilities are absent or limited.

C. Key Points

The following points are essential for conducting germination tests:

1. Laboratory germination tests are designed to provide the optimum conditions for germination and to determine the full germination potential of the seeds under these conditions.
2. The primary conditions to be considered are temperature, light, aeration, and moisture.
3. Rapid estimates of germination are just that – estimates; they are not as accurate as germination tests.
4. If more than 60 days are required for a germination test, analysts should use a rapid estimate for official testing.
5. Germination testing in the course of research may require different methods and equipment from official testing.
6. No matter how standardized the test prescriptions are, the judgment of the analyst must prevail in the laboratory.

D. Definition of Terms

Relevant terms in germination testing will be defined according to the glossary developed by the Seed Problems Project Group of the International Union of Forestry Research Organizations (IUFRO) (Bonner 1984a). These terms are defined as follows:

1. **Abnormal seedlings** – in seed testing, seedlings that do not possess all normal structures required for growth, nor show the capacity for continued development
2. **Filled seed** – a seed with all tissues essential for germination
3. **Germination** – resumption of active growth in an embryo, which results in its emergence from the seed and development of those structures essential to plant development
4. **Germination capacity** – proportion of a seed sample that has germinated normally in a specified test period, usually expressed as a percentage (synonym: germination percentage)
5. **Germination energy** – proportion of germination that has occurred up to the time of peak germination, the time of maximum germination rate, or some preselected point, usually 7 test days. (The critical time of measurement can be chosen by several means.)
6. **Germination percentage** – (see germination capacity)
7. **Hard seeds** – seeds that remain hard and ungerminated at the end of a prescribed test period because their impermeable seedcoats have prevented absorption of water
8. **Peak germination** – the specific time when rate of germination is highest. It may be derived in many ways (see germination energy).
9. **Pretreatment** – any kind of treatment applied to seeds to overcome dormancy and hasten germination
10. **Purity** – proportion of clean, intact seeds of the designated species in a seedlot, usually expressed as a percentage by weight
11. **Sample, submitted** – the sample of seeds submitted to a seed-testing station
12. **Sample, working** – a reduced seed sample taken from the submitted sample in the laboratory, on which some test of seed quality is made
13. **Seedlot** – a specified quantity of seeds of reasonably uniform quality
14. **Seed quality** – a general term that may refer to the purity, germination capacity, or vigor of a seedlot
15. **Sound seed** – a seed that contains in viable condition all tissues necessary for germination
16. **Tolerance** – a permitted deviation (plus or minus) from a standard. In seed testing, the permitted difference between or among replicated measurements beyond which the measurements must be repeated
17. **Vigor** – seed properties that determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions.

E. Quality Evaluation

For satisfactory evaluation of germination, the following principles are fundamental:

1. Sampling must be good; tests describe the sample only.
2. Testing at standard, optimum conditions ensures that:

a. Absolute maximum potential of the lot is determined.
b. Standard conditions can be duplicated by all laboratories for test comparison.

F. Methodology
Satisfactory germination testing depends on proper methods:
1. **Pure seed component** – Only the pure seed component is used in the test (4 replications of 100 seeds each).
2. **Environmental conditions** – Temperature, light, moisture, and medium must be carefully controlled.
   a. Temperature requirements differ according to species. International Seed Testing Association prescriptions should be followed.
   b. Light requirements are also spelled out in the ISTA rules.
   c. The germination medium must be nontoxic; it can be either natural or synthetic material.
      (1) Natural materials include soil, sand, peat, and other organic materials.
      (2) Synthetic materials include blotters, paper towels, cellulose wadding (Kimpak®), filter paper, agar, and cloth.
3. **Moisture** – Excessive moisture is a common problem in many tests.
4. **Equipment** – Germination equipment must be dependable.
   a. Cabinet germinators
   b. Jacobsen tables
   c. Walk-in rooms
   d. Small containers (petri dishes and plastic boxes)
5. **Test Procedures**
   a. Pretreatment
      (1) Micro-organism/pathogen treatment.
      (2) Overcoming dormancy (delayed germination)
         (a) stratification (prechill)
         (b) chemical treatment (nitrates, hydrogen peroxide, and growth regulators)
         (c) scarification for hard seeds
   b. Placement of samples
   c. Counting
      (1) Define "germinated seed"
      (2) Count frequency; weekly is the minimum
      (3) Recognize abnormal seedlings; common abnormalities are albino seedlings, stunted roots, negative geotropism, "endosperm" collars, and necrotic areas
   d. Length of test
   e. Determining condition of ungerminated seeds
6. **Tolerance and retesting**
   a. Review the concepts for official testing.
   b. Analysts should be aware of other reasons for a retest:
      (1) Too much dormancy; additional prechill needed.
      (2) Too much fungal infection; increase distance between seeds on blotter or test in sand or soil.
      (3) Normal/abnormal distinction unclear.
      (4) Evidence of human error.

G. Additional Testing Considerations
1. Thermogradient plates
2. Greenhouse or nursery bed tests
3. Testing by weight (e.g., *Eucalyptus* and *Betula*)
4. *Quercus* and other large seeds can be cut in half

H. Reporting Results
1. **Germination capacity**
2. **Rate of germination**
   a. Germination energy
   b. Mean germination time (MGT)
   c. Time for a certain proportion of germination to occur (e.g., number of days for 50 percent of the seeds to germinate)
   d. Germination Value (GV)
   e. Peak Value (PV)

I. Practical review

J. Sources
For additional information, see Bonner 1984a, 1984b; Czabator 1962; Edwards 1987; International Seed Testing Association 1985, sect. 5, 5A, 11; Willan 1985, p. 202–227.

V. Rapid Tests: Cutting, Vital Stains, Excised Embyro, and Hydrogen Peroxide

A. Introduction
The standard for judging seed quality is always a germination test under optimum conditions. Under certain circumstances, however, germination tests are not possible, and so-called "rapid tests" must be used to estimate seed quality. When performed properly, rapid tests can furnish valuable information to seed users, analysts, and managers.

B. Objectives
1. Learn the different types of rapid tests and how to perform them.
2. Recognize the limitations of each test and when it should be used.

3. Examine the interpretation of test results.

C. Key Points

The following points are essential to perform rapid tests:

1. The cutting test is the quickest and simplest and can be extremely useful with fresh seeds.
2. Tests with vital stains reveal more than just potential germination, but interpretation is subjective.
3. X-ray radiography is the most expensive, but not necessarily the best, of the rapid tests. It is very effective for some situations.
4. Leachate conductivity is a new and promising method.

D. Use of Rapid Tests

Rapid tests are used when one of the following conditions occurs:

1. **60-day rule of ISTA**—If a germination test requires more than 60 days to complete, then a rapid test should be used.
2. **Requested by user**
3. **Seed supply is limited**
4. **A quality check is needed during collection**
5. **There are other test objectives**

E. Sampling

The same sampling principles and precautions apply for rapid tests as for standard germination tests.

F. Test Methods

There are six rapid tests that have applications with tree seeds.

1. **Cutting**
   a. Technique: Seeds are cut in half lengthwise and all tissues are examined.
   b. Evaluation: Good seeds are firm, with good color.
   c. Advantages
      (1) Quickest and cheapest
      (2) Can be performed in the field
      (3) Is accurate on fresh seeds
   d. Disadvantages
      (1) Has size limitations
      (2) Produces poor results with stored seeds
      (3) Is a destructive test
2. **Vital stains**
   a. Technique: Embryo and storage tissues are exposed by cutting and then stained. Location and intensity of staining indicate viable or dead tissue.
   b. Stain options:
      (1) Tetrazolium chloride (TZ) (most widely used) stains live tissues red.
      (2) Indigo carmine stains dead tissues blue.
      (3) Selenium or tellurium salts.
   c. Evaluation (TZ only):
      (1) Sound tissue should stain carmine.
      (2) "Topographic stain" analysis is the most accurate, but it is the most difficult to standardize.
      (3) The ISTA (1985) prescribes TZ for certain dormant species.
   d. Advantages
      (1) Fast, stains can be read within 24 hours
      (2) Inexpensive
      (3) Equipment needs are simple
   e. Disadvantages
      (1) Labor-intensive
      (2) Difficult to obtain uniform penetration of the stain
      (3) Difficult to interpret the stain
      (4) Requires practice and experience
      (5) Destructive test
3. **Excised embryo**
   a. Technique: Seeds are cut open, and the embryos are incubated in dishes.
   b. Evaluation
      (1) Viable seeds are green and white, with some growth.
      (2) Nonviable seeds are dark or moldy, with no growth.
   c. Advantages
      (1) Simple equipment needs
      (2) Actual seed performance is tested
      (3) Easy to evaluate
   d. Disadvantages
      (1) Labor-intensive
      (2) Requires practice for proper excision
      (3) Slow (10 to 14 days)
      (4) Destructive test
4. **Hydrogen peroxide**
   a. Technique: Seedcoats are cut to expose the radicle, and the seeds are incubated in 1-percent hydrogen peroxide. Radicle growth is measured.
   b. Evaluation: Based on radicle growth.
   c. Advantages
      (1) Inexpensive test
      (2) Partially objective
      (3) Simple preparation
   d. Disadvantages
      (1) Not practical for very small seeds
      (2) Tested only on conifers
      (3) Destructive test
      (4) Slow (7 to 8 days)
5. **X-ray radiography**
   a. Technique: Intact seeds are exposed to soft x rays, and the images that are captured on film are examined.
   b. Evaluation: Evaluation is very subjective.

c. Advantages
(1) Fast
(2) Provides a permanent image
(3) Nondestructive
d. Disadvantages
(1) Equipment is expensive
(2) Extensive training is required
(3) Interpretation is subjective

6. **Leachate conductivity**
a. Technique: Seeds are leached in deionized water for 24 to 48 hours; electrical conductivity of the leachate is then measured.
b. Evaluation: Relationship of conductivity to germination must be established for each species.
c. Advantages
(1) Requires no expensive equipment
(2) Fast and simple
(3) Objective measurement
(4) Nondestructive
d. Disadvantages
(1) Indirect measurement of seed quality
(2) Unknown factors still cause trouble

G. Sources
For additional information, see International Seed Testing Association 1985, annex to chap. 6, app. B; Leadem 1984; Willan 1985, p. 221–226.

VI. Rapid Tests: X Rays and Leachate Conductivity

A. Introduction
Like other rapid tests, radiography offers a quick estimate of seed quality when there is no time for a complete germination test. The application of x-ray radiography in seed science is one of the few technologies that originated with tree seeds instead of agricultural seeds. It has not yet fulfilled its early promise, but there are many applications with seeds. Many rapid estimates of seed quality have major drawbacks: high cost, subjective interpretations, excessive time, etc. The leachate conductivity method offers a test that meets all requirements: low cost; fast, objective measurements; easy procedures; and nondestructive. Although relatively new, it shows great promise.

B. Objectives
1. Review x-ray theory, and see how x rays can be used in seed radiography.
2. Learn the principles of seed radiograph interpretation.
3. Examine the physiological basis for leachate testing.
4. Learn the leachate methodology.
5. Recognize the advantages and the disadvantages of both techniques.

C. Key Points
The following points are essential to an understanding of these two methods:
1. Many types of seed damage can be detected by x-ray testing.
2. Embryo development can be measured precisely, but exact correlations with germination are not possible.
3. The use of contrast agents can increase the amount of information obtained from radiographs; however, many of these agents kill the seeds.
4. Many special radiographic techniques are available, but most require equipment associated with medical x-ray technology.
5. As seeds deteriorate, cellular membranes are damaged, allowing the leaching of many substances from the seeds.
6. Many chemical groups can be detected, but electrolytic activity is the easiest to measure.
7. Good estimates of quality are possible with many species, but germination tests are still preferred as the standard measurement of seed quality.
8. The conductivity method is promising, but more research is needed.

D. X rays
1. **Theories**
a. X rays are electromagnetic energy of very short wavelengths. X rays penetrate materials that absorb or reflect light, and are themselves absorbed by the target object.
b. Radiographs are pictures of the object formed by the x rays that pass through the object and strike a photographic material.
(1) Radiograph quality is defined by contrast, density, and definition of the image.
(2) Quality is controlled by kilovoltage (kV), milliamperage (mA), exposure time, focus-film-distance (FFD), and object-film-distance (OFD).

2. **Methods**
a. Equipment: Several types of x-ray equipment are available commercially.
b. Film: Several film choices are available, including
(1) Conventional film

(2) Polaroid film
(3) Radiographic paper

c. Contrast agents: Contrast agents are used to increase density of certain seed tissue images on the radiograph.
(1) Aqueous agents are primarily solutions of heavy cation salts (e.g., barium chloride and silver nitrate).
(2) Vaporous agents: chloroform or other halogen derivatives of alkanes.

d. Safety is an important aspect of seed radiography.

3. **Special Techniques** – Mainly for research application, they include:
a. Stereoradiography
b. Tomography
c. Xeroradiography

4. **Applications in seed testing** – X rays were first used on seeds in Sweden in 1903.
a. The most effective uses are:
(1) Determining seed anatomy
(2) Determining insect damage
(3) Determining mechanical damage
b. X rays have limited usefulness in determining viability.

E. Leachate Conductivity

1. **Major points** – As seeds deteriorate, substances can be leached in proportion to the degree of deterioration. Sugars, amino acids, and electrolytes are just some of the materials that can be measured.

2. **Techniques** – Leachate conductivity can be measured in two ways:
a. Multiple-seed analyzers
(1) Advantages
(a) Fast
(b) Receives input from individual seeds
(c) Data are printed on paper tape
(d) Some models can calculate statistics
(2) Disadvantages
(a) High cost (US $6,500)
(b) Some equipment not reliable
(c) Influence on the conductivity/germination relationship unknown
b. Single probe techniques
(1) The ISTA handbook on vigor testing (Perry 1981) includes this method for peas.
(2) Advantages
(a) Fast
(b) Inexpensive equipment
(c) Completely objective
(d) Accuracy for some species within 10 percent of germination
(3) Disadvantage: Some factors have an unknown effect.

F. Sources
For additional information on x rays, see Vozzo 1978, 1988; Willan 1985, p. 224–226. For additional information on leachate conductivity, see Bonner 1991a, Perry 1981.

VII. Vigor Tests

A. Introduction
Standard germination tests do not adequately measure the ability of seeds to germinate and produce normal seedlings under field conditions because germination tests are conducted in the laboratory under optimum conditions. Such conditions are seldom encountered in the field, so germination and emergence may be much lower than in the laboratory. Therefore, a more sensitive measurement of seed quality has been sought by those concerned with the planting quality of a seedlot. This measurement of seed quality has been referred to as seed vigor. Seed vigor tests add supplemental information about the quality of seeds to information obtained through other tests.

B. Objectives
1. Learn the concept of seed vigor and realize how it can help the seed users.
2. Become familiar with the types of seed vigor tests and know which ones are most suitable for tree seeds.

C. Key Points
The following points are essential to an understanding of vigor tests:
1. Vigor is a seed quality that may or may not be indicated by a standard germination test.
2. Vigor is most important under adverse field conditions, and it can also indicate the storage potential of a seedlot.
3. Vigor tests usually involve either direct or indirect measurements.
4. For many tree seeds, rate of germination is the best expression of vigor.

D. Definition of Terms
1. **Vigor**
a. Association of Official Seed Analysts: "Those seed properties which determine the potential for rapid, uniform emergence, and development of normal seedlings under a wide range of field conditions" (AOSA 1983).
b. International Seed Testing Association: "The sum of the properties which determine the potential level of activity and performance of the seed or seedlot dur-

ing germination and seedling emergence" (Perry 1981).

c. International Union of Forestry Research Organizations: "Those seed properties which determine the potential for rapid, uniform emergence, and development of normal seedlings under a wide range of field conditions" (Bonner 1984a).

2. **Seed quality**—"A general term that may refer to the purity, germination capacity, or vigor of a seedlot" (Bonner 1984a).

E. Seed Vigor Concepts

1. **Physiological quality**—Seedlots vary tremendously in physiological quality. This is exemplified by the different rates of germination within a seedlot, the variation in the growth rates and sizes of seedlings produced, and the ability of some seeds to produce seedlings under adverse conditions while others do not. The physiological quality of seeds is commonly called seed vigor.

2. **Physiological maturity**—Seeds reach their maximum germination capacity and vigor during the maturation process at their maximum dry weight, or the "physiological maturity" stage. Once physiological maturity has been reached, deterioration begins and continues until the death of the seed. The process cannot be stopped, but the rate of deterioration can be controlled to some extent. Different seeds decline in vigor at different rates.

3. **Deterioration**—Seed vigor declines more rapidly than does the ability to germinate. The first sign of deterioration is a loss of vigor. Thus, a seed may germinate even though some of its physiological functions may have been impaired. The ability to produce seedlings under stress conditions and the growth and yield of plants may be affected as vigor declines. Vigor is thus a more encompassing measurement of seed quality than the standard germination test.

4. **Strategy**—The general strategy in determining seed vigor is to measure some aspect of the seed performance or condition that reflects the stage of deterioration or genetic deficiency. Developing a good test for this strategy is not easy. A practical seed vigor test should:

a. Be reproducible
b. Be easily interpreted
c. Indicate field performance potential
d. Take a reasonable length of time
e. Not require expensive equipment
f. Not require extensive training

F. Common Seed Vigor Tests

Vigor tests can be grouped into four categories:

1. **Seedling growth and evaluation**
   a. Seedling vigor classification
   b. Seedling growth rate
2. **Stress tests**
   a. Accelerated aging
   b. Cold test
   c. Cool germination test
   d. Osmotic stresses
   e. Methanol treatment
3. **Biochemical tests**
   a. Tetrazolium chloride (TZ) staining
   b. Adenosine triphosphate (ATP) activity
   c. Glutamic acid decarboxylase activity (GADA)
   d. Oxygen uptake (respiration)
   e. Leachate tests
      (1) Sugars
      (2) Amino acids
      (3) Electrolytes
4. **Germination data**
   a. Mathematical modeling of germination response
      (1) Normal distribution
      (2) Polynomial regressions for curve fitting
      (3) Logistic function
      (4) Probit transformation
      (5) Weibull function
   b. Germination rate
      (1) Early counts
      (2) Percentiles
      (3) Mean germination time (MGT)
      (4) Germination value (GV) and Peak value (PV)

G. Recommendations For Tree Seeds—The following tests have the most potential for tree seeds:

1. Germination rate parameters
2. Seedling growth tests
3. Tetrazolium staining for large seeds
4. Accelerated aging
5. Leachate conductivity

H. Sources

For additional information on vigor tests, see Association of Official Seed Analysts 1983; Blanche and others 1988; Bonner 1986b; Perry 1981; Willan 1985, chap. 9.

Protection

I. Insects

A. Introduction

Insects are one of the greatest destroyers of tree fruits and seeds. They reduce both quality and quantity of seeds and affect angiosperms and gymnosperms equally. Damage is done through all reproductive stages, from developing buds to cleaned seeds in storage. Losses to seed insects are huge, and much is yet to be learned about their complete role in the reproductive cycle of woody plants.

B. Objectives

1. Learn the orders of insects that cause the most damage to tree seeds and the species they attack.
2. Recognize the types of injury that insects cause.
3. Learn some methods of insect control and management.

C. Key Points

The following points are essential in protecting seeds from insects:

1. Insects of the orders Hymenoptera, Diptera, Lepidoptera, Hemiptera, Coleoptera, Homoptera, and Thysanoptera do the most damage to flowers, fruits, and seeds of woody plants.
2. Damage ranges from causing reproductive structures to abort to causing loss of seeds in storage.
3. General types of damage include:
   a. Destroying the seeds only, Hymenoptera (wasps).
   b. Forming galls and mine scales, Diptera (flies).
   c. Free feeding, Lepidoptera (moths).
   d. Consuming endosperm, Hemiptera (true bugs).
   e. Mining cone axes, Coleoptera (beetles).
   f. Causing cone abortion, Homoptera (aphids, etc.) and Thysanoptera (thrips, etc.).
4. Control methods depend on identifying and knowing the insect's life cycle and the host-plant relationship.
5. Some methods for reducing damage are preventive measures, insecticides, natural biological control agents, and proper management techniques.

D. Damage

1. **General concepts**
   a. Insects reduce seed production by infesting buds, flowers, cones, and seeds.
   b. The most damaging insects are largely restricted to six orders: Lepidoptera (moths and butterflies), Diptera (flies), **Coleoptera (beetles), Hymenoptera (wasps), Hemiptera (true bugs), and Thysanoptera (thrips).**
2. **Specific concepts**
   a. Coleoptera (beetles) are the most damaging group in arid and semiarid zones.
      (1) Bruchidae (bruchid beetles) are the most important by far for Leguminosae; e.g., beetles in the genera: *Amblycorus, Bruchidius,* and *Caryedon*.
      (2) Curculionidae (weevils) lay their eggs on developing fruits:
         (a) *Conotrachelus*
         (b) *Curculio* and *Conotrachelus*
         (c) *Thysanocnemis*
         (d) *Nanophyes*
         (e) *Apion ghanaense*
   b. Lepidoptera (moths and butterflies) damage stored seeds:
      (1) Pyralidae
      (2) *Melissopus* and *Valentinia*
      (3) *Agathiphaga*
      (4) Gelechiidae
   c. Hemiptera (true bugs) feed on seeds with specialized sucking mouth parts:
      (1) Coreidae attack *Erythrina* seeds in India and some *Acacia* species in Africa
      (2) Pentatomidae
   d. Hymenoptera (wasps) feed on seeds:
      (1) Torymidae (*Megastigmus* spp.) larvae feed on *Pinus, Abies,* and *Pseudotsuga*
      (2) Eurytomidae (*Bruchophagus*)
   e. Homoptera (aphids, cicadas, and scales) are not a major threat to seeds.
   f. Thysanoptera (thrips) cause some damage to tree seeds.

E. Controlling insects

Control measures must be guided by the species and ecology of the insect.

1. **Prevention** – The insect may be prevented from reaching the seeds.
2. **Chemical control** – Includes foliar sprays, systemic poisons, light traps, chemical traps, and carbon dioxide.
3. **Natural enemies** – The target insect's life cycle and history should reveal its natural enemies.
4. **Collection practices** – Collecting good seeds is the first step in keeping down losses incurred in storage.

F. Sources

For additional information, see Cibrian-Tovar and others 1986, Johnson 1983, Schopmeyer 1974, Southgate 1983.

II. Pathogens

A. Introduction

Pathogenic organisms (fungi, bacteria, and viruses) cause great economic losses. Not only are seeds the victim of pathogens, but they also are passive carriers (vectors) of pathogens that may not directly affect the seeds but may endanger other organisms. This fact is the basis of plant quarantine regulations that include seeds in the import and export restrictions on plant material.

B. Objectives

1. Learn the major types of seed pathogens and the typical damage that they cause.
2. Identify steps to decrease losses to seed pathogens.
3. Review documented occurrence of micro-organisms associated with tree seeds.

C. Key Points

The following points are essential to preventing seed pathogens:

1. The major disease-causing organisms are fungi, bacteria, and viruses.
2. All tree seeds carry micro-organisms, primarily on the surface of their seedcoats.
3. All seed micro-organisms are not pathogenic; some may even be beneficial.
4. Pathology of tree seeds has not been studied extensively; much work remains to be done.

D. Types of Pathogens

1. **Viruses**
   a. Viruses account for seven kinds of seed damage:
      (1) Abortion of seeds
      (2) Flower sterility
      (3) Seedcoat wrinkling
      (4) Shriveling
      (5) Chalky endosperm
      (6) Staining
      (7) Necrosis
   b. In legumes, embryo-borne viruses reduce viability.
   c. A high incidence of triploidy can result from viral infection.
   d. Market value of seeds can be reduced.
   e. A virus can outlive the seed.
2. **Bacteria** – Bacterial infections account for four kinds of seed damage:
   a. Abortion
   b. Rot
   c. Discoloration
   d. Slime disease
3. **Fungi** are a serious threat to seed health simply because of the great numbers of representative species known as seed pathogens. Fungi account for eight kinds of seed damage:
   a. Abortion
   b. Shrunken seeds and reduced seed size
   c. Rot
   d. Sclerotization and stromatization
   e. Necrosis
   f. Discoloration
   g. Lowered germination capacity
   h. Physiological alterations

E. Control Mechanisms

Seed pathogens can be controlled by reducing infection and by treating seeds in laboratories, storage facilities, and nurseries.

1. **Infection reduction** – Infections in orchards can be reduced by:
   a. Locating seed orchards in areas of low infection risk
   b. Removing alternate host plants
   c. Sanitizing orchards
   d. Applying fungicides
   e. Using good cone- and fruit-handling methods
2. **Seed treatment in laboratories**
   a. Surface sterilization
   b. Fungicides
   c. Hot water soaks
3. **Seed treatment in storage**
4. **Seed treatment in nurseries**
   a. Damping-off
   b. Seedling diseases

F. Micro-organisms Found on Tree Seeds

See the checklist of Anderson (1986a).

G. Sources

For additional information, see Anderson 1986a, International Seed Testing Association 1966, Neergard 1977, Sutherland and others 1987.

Basics for Nurseries

I. Production Systems

A. Introduction

This course is not intended to cover all aspects of nursery establishment and management. However, a few nursery problems involve seeds and seed management practices. The type of nursery system, size of the nursery, and location are important for seeds. It was once believed that all seedling production and planting in the Tropics had to be done in containers. This is not true; in general, however, bare-root production systems predominate in the Temperate Zones and container production systems predominate in the Tropics.

B. Objectives

1. Recognize different nursery systems and the conditions most favorable for each.
2. Learn the relationship of nursery systems to national seed program management.
3. Review basic seed technology for sowing in each system.

C. Key Points

The following points are essential in understanding seed basics for nurseries:

1. Bare-root systems are more common in Temperate Zones; container systems are more common in the Tropics.
2. Bare-root production is possible in the Tropics with some pines and for stump production of selected species.
3. In container systems, large seeds are usually sown directly in containers, while small seeds are sown in germination beds or trays and transplanted (pricked out).
4. Tray mobility is an advantage in caring for and protecting young seedlings.
5. In small nurseries, seed treatments for germination are usually done by hand.

D. Core Material

1. Type of Nursery
   a. **Bare-root systems**
      (1) Are suitable with large-scale planting programs.
      (2) Can produce seedlings or stumps.
      (3) Require same-day planting in tropical environments.
      (4) Used with *Pinus caribaea* in Venezuela and stump plantings of *Gmelina, Dalbergia sissoo,* and *Cassia siamea.*
   b. **Container production systems**
      (1) Are preferred in most tropical locations because:
         (a) They can be small, labor-intensive operations.
         (b) Containerized seedlings can stand the transport stress.
      (2) System options include:
         (a) A large centralized nursery with production of 0.5 to 1.0 million seedlings
         (b) Numerous small nurseries with production of 10,000 to 100,000 seedlings
      (3) This system can be used for "wildings."
   c. **Seed program considerations**
      (1) In a large, centrally located nursery, seed cleaning and storage should be located nearby.
      (2) In small, dispersed nurseries, cleaning and short-term storage should be in a regional center.
      (3) In small nurseries, much seed collection, extraction, and cleaning are performed locally.
      (4) Localized collection forces the use of local seed sources.
      (5) For tropical recalcitrants, small local nurseries must be used to avoid viability loss in seeds.
      (6) A combination of approaches will probably evolve.
2. Bare-Root Production
   a. **Small seeds**—For small seeds, use mechanized sowing and culture.
   b. **Large seeds**—For large seeds, sow by hand.
   c. **Covering**
      (1) Small seeds—Press into the soil surface and cover with a light mulch (2 to 3 mm).
      (2) Large seeds—Place on their sides, press into the soil, and cover with 5 mm of soil.
3. Container Production

   In container production, either sow directly into containers, or sow in seedbeds or seed trays and transplant later (pricking out).
   a. **Sowing into containers**
      (1) Is good for the root systems.
      (2) Is used for
         (a) Large seeds that can be handled individually
         (b) Seedlots with expected high germination
      (3) Sustained sowing rates are shown in table 16.
      (4) Allows pricking out of "doubles."
      (5) Aims for one seedling per container.
      (6) Calculation of seed needs.

b. **Sowing into seedbeds or seed trays**
   (1) Concentrates germination in small areas.
   (2) Is used for
      (a) Seedlots with expected germination of less than 40 percent
      (b) Seedlots with slow germination
      (c) Species that have several seedlings per seed unit
      (d) Very small seeds
      (e) Scarce or expensive lots
   (3) Provides the advantages of seed tray mobility.
   (4) Follow these steps:
      (a) Sand:topsoil mix of 1:1.
      (b) Pure sand for *Pinus, Eucalyptus,* and others.
      (c) Press seeds into medium, barely cover with washed sand, and mulch lightly.
      (d) Monitor closely to maintain proper moisture level.
   (5) Sowing into seedbeds is most common.
      (a) Provide well-drained seed beds.
      (b) Broadcast small seeds, pressing them into soil, and covering lightly.
      (c) Protect from rodents.
      (d) Sow very small seeds by mixing seeds and fine sand.
   (6) Calculate sowing rates.

Table 16.—*Suggested sowing rates for seedling production in containers (Napier and Robbins 1989)*

Expected germination	Seeds per container
Percent	*Number*
80	1 or 2*
60–79	2
40–59	3
<40	use seedbeds

*Sow half the containers with one seed and half the containers with two seeds.

D. Sources

For additional information, see Lantz 1985, Liegel and Venator 1987, Napier and Robbins 1989, Willan 1985.

Seed Programs

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I. National Programs

A. Introduction

National seed programs are necessary to support national reforestation and afforestation efforts by ensuring an adequate supply of high-quality seeds of suitable species and sources. Countries of the Association of Southeast Asian Nations are losing over 1.2 million hectares of forest lands annually to other uses. Deforestation of "officially" designated forest lands in India has not been excessive since the 1950's (about 3 percent of the lands under the Forest Department), but more than 10 times this area of wastelands, small groves, etc., has been denuded. Within the framework of national programs, State or Provincial seed programs may also be needed.

B. Objectives

1. Learn the general functions of a national forest-seed program.
2. Examine possible administrative structures of a national program.
3. Examine an existing national program as a case study.

C. Key Points

The following points are important in national seed programs:

1. The primary function of a national forest-seed program is to ensure an adequate supply of suitable tree seeds.
2. National programs can serve many other important functions.
3. National programs should serve the needs of all tree planting: industrial wood plantations, watershed protection, social forestry plantings, agroforestry, etc.

D. Tree-planting activities should be served by a national forest-seed program.

1. **Industrial wood products**
2. **Fuelwood and charcoal**
   a. Village forests
   b. Individual landowners
   c. Commercial production
3. **Watershed protection**
   a. General protection
   b. Protection in specific areas (i.e., reservoirs, mine spoils, dune stabiliation)
4. **Windbreaks or shelterbelts**
5. **Urban planting**
6. **Wildlife habitat and food plantings**
7. **Agroforestry planting**
8. **Social forestry**
9. **Conservation of genetic resources**

E. Scope of the Program

1. **Population distribution**
2. **Physiographic characteristics**
3. **Available land area and ownership**
4. **Realistic annual goals**
5. **Seed storage needs**
6. **Use of indigenous species**

F. Species Choices

1. **Indigenous species and land races** – These may be best.
2. **Exotics** – Caution should be used with exotics.
3. **Seed source** – Provenance tests are needed.
4. **Natural plant succession should be followed.**

G. Administrative Structure – Many government agencies or ministries may be involved, such as:

1. **Forestry ministry levels**
   a. National
   b. Provincial or State
   c. Village or other local structures
2. **Comprehensive natural resource agencies**
3. **Agricultural agencies**
4. **Military departments**
5. **Division of responsibilities**
   a. Overall planning
   b. Seed acquisition and distribution – There may be a central location or regional centers for the following:
      (1) Collecting and cleaning
      (2) Testing
      (3) Storage
      (4) Certification
      (5) Record keeping
      (6) Sales to other countries
      (7) Sales within country
   c. Seedling production can be based in:
      (1) National or State nurseries
      (2) Village nurseries
      (3) Private nurseries (farmers)
      (4) Commercial nurseries
   d. Plantation care – Two factors must be considered:
      (1) Protection, primarily from animals, fire, and people
      (2) Measurement of survival and early growth
   e. Research – Many problems may require research, such as:
      (1) Seed problems
      (2) Species, site, and seed source evaluations

H. Critical Steps

Several critical steps in the planning process call for good decisions:

1. **Planting goals** – what, where, and how much?
2. **Availability of seed supply**

a. Indigenous species
b. Commercial sources

3. **Collection crews**
   a. Equipment and transport
   b. Training
   c. Legal obstacles
4. **Nursery administration**
   a. Site
   b. Personnel
   c. Equipment
5. **Collection goals**
6. **Seed centers** – National, State, or regional?

I. Other Considerations

1. **Continuity of operations**
2. **Training**
3. **Multiple functions**
   a. Some foresters also grow and distribute fruit trees.
   b. In some countries, only one seed laboratory is available to test both agricultural and tree seeds.
4. **International organizations** that can help in planning:
   a. ISTA – ISTA Secretariat
      Reckenholz, P. O. Box 412
      CH-8046 Zurich
      Switzerland
   b. IUFRO – IUFRO Secretariat
      Schonbrunn
      A-1131 Vienna
      Austria
   c. FAO – Forest Resources Development Branch
      Forest Resources Division
      Forestry Dept., FAO
      Via delle Terme di Caracalla
      I-00100 Rome
      Italy
   d. ICRAF – International Council for Research in Agroforestry
      P.O. Box 30677
      Nairobi
      Kenya

J. Case Study

K. Summary

The functions of a national seed center are:

1. Further develop taxonomy and aids to species identification.
2. Collect and disseminate data on the ecology of individual species, thus enhancing understanding of the performance of species.
3. Promote measures, as necessary, to conserve the genetic resources of important species.
4. Develop optimum seed collection strategies based on knowledge of breeding systems.
5. Maintain existing seed collections and ensure their future development as programs evolve to utilize promising species and provenances.
6. Assist collectors from other countries within the framework of national policy; some countries restrict collections by foreign nationals.
7. Provide information on the physical and physiological characteristics of seeds, and any diseases that might be borne by seeds.
8. Encourage quarantine practices that minimize the chances of domestic insects becoming established in other countries.
9. Disseminate information by providing appropriate training, symposia, and publications.
10. Disseminate seed samples for research or species trials to other institutions or countries on a cost or exchange basis.

L. Sources

For additional information see Gregg 1983, Hellum (in press), Robbins and Shrestha (in press), Rudolf 1974.

II. Seed Centers

A. Introduction

National forest-seed programs require some sort of national tree-seed center, institute, or laboratory. Dedicated facilities and some centralized authority are suggested for tree-seed centers. Their level of technology may vary with the country's needs, but these centers should serve as the focal point of seed activities.

B. Objectives

1. Learn the general functions of national tree-seed centers and how they support national seed programs.
2. Examine several options for center design.

C. Key Points

The following points are essential to seed center development:

1. The primary function of a seed center is to support the national forest-seed program.
2. Seed centers provide seed services, research on seed problems, training of seed workers, and extension activities for seed users.
3. Many countries will require regional or subcenters for efficient operation.

D. Functions

1. **Services**
   a. Coordinates seed collection
   b. Conditions seed collections
      (1) All operations at a main center

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(2) Drying and extracting at regional centers
c. Storage of seeds
(1) Operational storage
(2) Long-term storage of surplus stocks
(3) Very long-term storage
d. Testing
(1) National seed program collections
(2) Other in-country users
(3) Third-party testing
e. Certification

2. **Seed research**
a. Applied research
b. Basic research

3. **Training and extension**
a. Train seed collectors, analysts, and others
b. Extension programs for nursery workers and farmers

E. National or Regional Centers

1. **National centers** can be more responsive to political realities.
2. **Regional centers** can expand the scope of operations.
3. **Compromise**—National centers are best for storage, testing, and research; regional centers are good for collecting and cleaning.

F. Location Concerns

1. **Proximity to seeds**
2. **Transportation**
3. **Isolation**
4. **Technical help**
5. **Disaster potential**

G. Center Design

1. **Activity zones** include the following areas:
a. Loading dock
b. Drying area
c. Extraction equipment
d. Cleaning equipment
e. Conditioning equipment
f. Seed storage
g. Testing laboratory
h. Offices for records and supervision
i. Supply storeroom.

2. **Building design**—Suggested floor plans and designs are available from ISTA.

3. **Equipment**
a. Commercial sources are best, but much can be made locally.
b. Spare part sources are crucial.
c. Maintenance must be available.
d. Electrical supply must be dependable.

4. **Staffing**—Supervisors should have defined areas of work:
a. Director of the center
b. Collection supervisor
c. Extraction and cleaning supervisor
d. Testing supervisor
e. Inventory and shipping supervisor

5. **Training**—All staff members should be trained in their specialties by university staff, special short courses, or on-the-job training at an established center. If personnel change jobs, the new people must be trained immediately. The skills of long-time staff should be updated as new methods are developed.

III. Labeling and Certification

A. Introduction

When forest reproductive materials (seeds, seedlings, and vegetative propagules) are not collected or grown by the user, that user should have reasonable assurance of the identity and quality of the material he is buying. Many seed-labeling laws require detailed labeling to assure the buyer of the seeds' identity, purity, viability, and freedom from pests; i.e., the physiological quality of the seedlot. Certification is more than labeling required by seed laws; it is a statement about the genetic quality and identity of the seedlot.

B. Objectives

1. Understand the purpose of certification.
2. Identify the general elements of a certification program.
3. Describe the four certification categories used in the Organization for Economic Cooperation and Development (OECD) standards for international trade.

C. Key Points

The following points are essential to understanding labeling and certification of forest reproductive materials:

1. Certification is the guarantee by an officially recognized organization that forest reproductive materials of identified varieties have been grown, collected, processed, and distributed in a manner to maintain high quality and genetic identity.
2. A certification program requires a certification agency, a producer who wishes to sell certified material, records of the breeding program, certification standards, independent inspections, and certification labels.
3. The four certification categories used by OECD are:
a. source-identified (yellow tag)
b. selected (green tag)
c. untested seed orchards (pink tag)
d. tested reproduction material (blue tag)

4. Certification usually requires inspections of the production unit prior to pollination, a crop inspection before harvest, inspections during the collection-to-storage phases, and inspections at the time of packaging materials for sale.

D. Certification
   1. **Definition** – Certification is the guarantee of character and quality of reproductive materials by an officially recognized organization.
   2. **Purpose** – Certification is more than just labeling. Its purpose is to maintain and make available to the public high-quality seeds and propagating materials of superior crop plant varieties.
   3. **International aspect** – An international scheme for certifying forest reproductive material has been developed by OECD.

E. Definition of Terms
   The following definitions are for terms used in the OECD Scheme (Organization for Economic Cooperation and Development 1974):
   1. **Forest reproductive material**
      a. **Seeds:** cones, fruits, and seeds intended for the production of plants
      b. **Parts of plants:** stem, leaf, and root cuttings, scions and layers intended for the reproduction of plants
      c. **Plants:** plants raised by means of seeds or parts of plants; also includes natural regeneration
   2. **Clone** – a genetically uniform assemblage of individuals derived originally from a single individual by vegetative propagation, such as by cuttings, divisions, grafts, layers, or apomixis
   3. **Cultivar** – an assemblage of cultivated individuals, which is distinguished by any characters (morphological, physiological, cytological, chemical, or others) significant for the purposes of agriculture, forestry, or horticulture and which, when reproduced (sexually or asexually), retains its distinguishing features
   4. **Provenance** – the place in which any stand of trees is growing; the stand may be indigenous or nonindigenous. (This is the location of the seed source.)
   5. **Origin** – for indigenous stands of trees, the origin is the place in which trees are growing; for nonindigenous stands, the origin is the place from which the seeds or plants were originally introduced
   6. **Designated authority** – an organization or institution designated by and responsible to the government of a country participating in the OECD scheme for the purpose of implementing the rules of the scheme on its behalf

F. General Elements of a Certification Program
   1. **Designated authority** – The designated authority must have legal standing.
   2. **Producer** – There must be qualified producers.
   3. **History of the material** – These data cover provenance, seed source, and breeding history.
   4. **Supervised production** – The designated authority does this.
   5. **Standards** – The material must meet minimum standards.
   6. **Certification labels** – Labels are attached to all products.

G. Standards for Certification
   1. **Certification classes** – Forestry programs typically use the following OECD standards:
      a. Source-identified reproductive material (yellow tag). Conditions are:
         (1) Seed source and/or provenance must be defined.
         (2) Seeds must be collected, processed, and stored under inspection.
      b. Selected reproductive material (green tag). Conditions are:
         (1) Isolated
         (2) Normal variation
         (3) Sufficient size
         (4) Sufficient age and stage of development
         (5) Phenotypic superiority
      c. Material from untested seed orchards (pink tag).
      d. Tested reproductive material (blue tag).
   2. **Seed collection zones** have special features:
      a. They are delimited by administrative and geographic boundaries.
      b. Boundaries and reference numbers of seed collection zones should be established and published.
      c. Seed collection zones are necessary for "source-identified reproductive material."
   3. **Other requirements of certification**
      a. The originator, developer, owner, agent, or producer must request certification and furnish:
         (1) Name of the variety
         (2) Statement of the variety's origin
         (3) Detailed description of characteristics that distinguish the variety

(4) Evidence of performance
(5) Statement on the suggested area of adaptation

b. Inspections may include:
(1) Initial field inspections
(2) Mature crop inspections
(3) Inspections during collection, conditioning, and storage
(4) Inspection at the time of packaging for sale

c. Fees are paid by producer to support the system.

H. Other Documentation

1 **Labels** – Some countries or other political entities require labels for commercial sales with identity (species), purity, germination, etc., on the labels. No certification is implied.

2. **Phytosanitary certificate** – Phytosanitary certification is required by most countries to stop the spread of insects and pathogens. It certifies that the seeds have been inspected and/or treated.

I. Sources

For additional information, see Bonner 1981a, Organization for Economic Cooperation and Development 1974, Rudolf 1974.

IV. Germplasm Conservation

A. Introduction

Loss of forests around the world is widely deplored for many reasons. One consequence of deforestation is the loss of valuable germplasm that could be used in artificial regeneration and future breeding programs. The Food and Agriculture Organization (FAO) lists more than 300 tree species or provenances as endangered. Fortunately, there are steps that can be taken to conserve this germplasm.

B. Objectives

1. Recognize the consequences of excessive loss of germplasm of forest trees.
2. Learn the strategies available to conserve germplasm.

C. Key Points

The following points are essential to understanding germplasm conservation:

1. The ideal practice would be extensive in situ preservation.
2. Ex situ conservation is widely practiced already, but "passport dat " on planted material need to be maintained.
3. Seed storage can play a critical role in germplasm conservation.
4. National programs of conservation should be carefully planned and established.

D. Importance of the Problem

1. **Deforestation**
2. **Insect and disease losses**
3. **Global climate changes**
4. **Endangered species**

E. Available Technologies for Conservation

The following strategies are options for germplasm conservation:

1. **In situ conservation**
2. **Ex situ conservation**
3. **Conventional seed storage**
4. **Cryogenic storage**
5. **Storage of pollen**
6. **Micropropagation tissues**

F. Current Efforts

The following organizations engage in some germplasm conservation (table 17):

1. Food and Agriculture Organization (FAO)
2. International Board for Plant Genetic Resources (IBPGR)
3. Central America and Mexico Coniferous Resource Cooperative (CAMCORE)
4. Oxford Forestry Institute (OFI)
5. Danish International Development Agency (DANIDA)
6. Centre Technique Forestier Tropical (CTFT)
7. Commonwealth Scientific and Industrial Research Organization (CSIRO)
8. Many other countries have national seed storage facilities

G. Recommendations for Action

1. **Increased efforts in in situ conservation**
2. **International efforts for more ex situ conservation plantings**
3. **More research on conventional seed storage**
4. **Increased research with recalcitrant seeds**
5. **Establish more seed banks**

V. Applied Research

A. Introduction

Many seed problems can be solved locally without sophisticated research equipment that is costly to acquire and operate. Some investigations furnish answers without statistical treatment; others need statistical work to demonstrate their reliability. Simple designs are usually satisfactory in seed work, including completely randomized treatments and factorials. The main requirements are curiosity and dedication.

B. Objectives

1. Learn a few principles of simple research studies.

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Table 17.—*Some major international seed storage centers**

Center	Country	Approximate size of collection: Species	Approximate size of collection: Sources	Reference
		----- *Number* -----		
United States Forest Tree Seed Center	United States	67	197	Karrfalt (1985)†
National Seed Storage Laboratory	United States	18	41	Bass (1985)†
Petawawa National Forestry Institute	Canada	118	2,130	Janas (1984)
DANIDA Forest Seed Centre	Denmark	46	187	Anonymous (1985)
CSIRO Tree Seed Centre	Australia	900	4,000	Turnbull and Doran (in press)
OFI Oxford, UK	United Kingdom	‡	‡	‡
Banco Latinoamericano de Semillas Forestales	Costa Rica	153	308	Anon. (1983)
Banco de Semillas COHDEFOR	Honduras	4	46	Gustavo (1985)†

*Bonner, F.T. 1986. Unpublished report. On file with: USAID Science and Technology Office, Washington, DC. [Number of pages unknown].

†Personal communication from center directors.

‡Data not available.

2. Review case study examples of applied seed research.

C. Key Points

The following points are essential to applied seed research:

1. Problems can often, but not always, be solved with simple tests and experiments.
2. Standard procedures are always used when they are available; e.g., ISTA (1985) rules for germination testing.
3. Treatments are always replicated with several seed sources or in different seed years.
4. Limitations of the procedures in use must be recognized; e.g., electric seed moisture meters cannot be accurate to 0.1 percent.

D. General Considerations

1. **Replication**—The "standard" is 4 replicates of 100 seeds each (typically designated 4 × 100).
2. **Documentation**—Complete records are essential.
3. **Statistics**
   a. Studies must be designed to allow statistical analysis.
   b. Simple designs are used whenever possible.
   c. Common sense cannot be replaced with statistics.
4. **Publication**—Good results should be published.

E. Case Studies

1. **Maturity indices of fruits or seeds**—determine by:
   a. Using a minimum of five trees.
   b. Sampling for a reasonable time period.
   c. Collecting 10 to 15 fruits per tree.
   d. Taking color photographs if possible.
   e. Testing for the best parameters:
      (1) Size (length and diameter)
      (2) Weight (wet and dry; dried at 103 °C for 15 to 24 hours)
      (3) Moisture content
      (4) Germination
      (5) Chemical analyses
   f. Collecting data and plotting means on a time scale.
   g. Repeating at least twice to cover three seed crops.
2. **Extracting and cleaning methods**
   a. Possible tests include:
      (1) Sun drying vs. shade drying
      (2) Hand extraction vs. machine extraction

(3) Any mechanical action vs. hand cleaning
(4) Determining seed size effects by sizing into three groups and testing germination
(5) Dewinging vs. sowing winged seeds

b. Each treatment should be replicated 5 times; each replicate is tested with 4 samples of 50 seeds each.
c. Unusual results are always retested.
d. Suggested statistical designs are "t" tests for two treatments and complete randomization for more than two treatments.

3. **Pretreatment for germination**
   a. Possible methods to test include:
      (1) Hand scarification vs. mechanical scarification
      (2) Hot vs. cold water soak
      (3) Stratification time and temperature
      (4) Chemical stimulation
   b. The same general directions as described in "2.b" through "d" above apply in these tests also.
4. **Storage conditions**
   a. Possible tests include:
      (1) Room temperature vs. refrigerated conditions
      (2) Different refrigeration temperatures
      (3) Seed moisture levels
      (4) Type of storage containers
   b. Replicates should be large enough to allow sampling over time.
   c. Frequency of testing for orthodox seeds is 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 years, and for recalcitrant seeds, frequency is 1, 2, 4, 8, 12, 18, and 24 months, then every 6 months thereafter.
   d. At least four replicates should be used.
5. **Testing for recalcitrance**—A good test for recalcitrance is:
   a. Bring the seedlot to full imbibition.
   b. Start drying with at least two rates (slow and fast).
   c. Take periodic samples for moisture content and germination.
   d. Maintain the drying range from full imbibition to 10-percent moisture or until death of the seeds.
   e. Designate seeds that cannot be dried below 20 percent as recalcitrant.
   f. Repeat this test to confirm recalcitrance; never trust just one measurement cycle. Tests of additional seedlots are desirable.
   g. Check chilling injury at 0 to 5 °C by exposing fully imbibed seeds to this temperature for 24 hours.
   h. Keep statistics in perspective. Realize that they are not as important as common sense in interpretation of results.

Laboratory Exercises

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Exercise 1 – Seed Structure

Objective:

To learn basic seed structures and their function in important seed types.

Methods:

1. Presoak seed samples in tapwater at room temperature (or 27 °C) for 15 to 24 hours. The imbibition will soften tissues and facilitate dissection.
2. Using a knife, clippers, or a single-edged razor blade and depending on type of seeds, carefully cut the seeds in one of two different ways:
   a. cross section (transverse)
   b. lengthwise (longitudinal)
   Several cuts may be necessary to expose the embryo and other internal tissues.
3. Examine the tissues exposed by the cuts and label them on freehand sketches of the cut material. Determine which tissues are for embryo protection, storage of food reserves, etc. Look for abnormal structures, insect damage, etc.
4. On at least one seed of each species, try to remove the embryo without damage, sketch it, and label the parts.

Supplies:

Clippers (or knives), single-edged razor blades, dissecting needles, a small magnifying glass (or hand lens), pencil and paper, and seed samples of five tree species.

Exercise 2 – Seed Crop Estimation

Objective:

To predict seed crops in advance of collection by estimating the number of:

1. Good seeds per fruit
2. Fruits per tree

Methods:

1. Good seeds per fruit
   a. Choose a multiple-seed fruit and collect 15 fruits prior to maturity.
   b. Cut fruits in half lengthwise and count good seeds visible on each half.
   c. Dry the fruit halves in an oven (40 to 50 °C) to extract seeds and to obtain actual counts of good seeds.
   d. Calculate regression equations to predict total seeds from fruit cross-section counts.
2. Fruits per tree – Visit nearby trees and estimate fruit crops by:
   a. Total count
   b. One-fourth crown count
   c. Sample branch count
   d. Any other known ways
3. Combine results of both methods to estimate size of the seed crop.

Supplies:

Cone cutters or sharp blades, an oven, drying containers, and binoculars.

Exercise 3a – Cone Drying and Seed Extraction (Central America)

Objective:

To learn how to calculate seed and fruit needs for a planting program.

Assumptions:

Area to plant – 2,000 hectares (ha) at 1,700 trees per hectare
Species – *Pinus caribaea*

1. All moisture contents are percentage of wet weight.
2. There are 800 closed cones per hectoliter (hL) (40 kg).
3. Moisture content of closed cones is 40 percent.
4. Cones double in size when open.
5. Yield averages 400 grams (g) of pure seeds per hectoliter of closed cones.
6. There are 68,200 seeds per kilogram (kg).
7. Laboratory germination is 80 percent; 50 percent of the germinated seeds produce plantable seedlings.
8. Cones are put into the kiln when they reach 25-percent moisture content.
9. Drying trays hold 0.5 hL of closed cones; each stack of eight drying trays holds 4.0 hL; eight stacks can fit in the kiln at once.
10. It takes 12 hours to dry a full charge to the 10-percent cone moisture needed for the cones to open fully.
11. It takes 700 kilocalories (Kcal) to heat 1 hL of cones for 1 hour.
12. Fuel value for wood of *Casuarina equisetifolia* is 4,950 Kcal per kilogram; for *P. caribaea* cones, 4,500.
13. Open *P. caribaea* cones weigh 104 g per liter (L).

Questions:

1. How many cones must be collected to meet the planting goal?
2. How much total moisture must be lost in predrying (prior to entering kiln)?
3. How many drying stacks will be needed to predry everything at once?
4. How many kiln charges will be needed?
5. How long will it take to open all cones?
6. How much fuel will be needed with *C. equisetifolia* wood? with *P. caribaea* cones?
7. Have enough cones been collected to heat the kiln?

Exercise 3b – Cone Drying and Seed Extraction (India/Pakistan)

Assumptions:

Area to plant – 2,000 ha at 1,700 trees per hectare
Species – *Pinus roxburghii*

1. All moisture contents are expressed as a percentage of wet weight.
2. There are 400 closed cones per hectoliter (hL) (40 kg).
3. Moisture content of closed cones is 40 percent.
4. Cones double in size when open.
5. Yield averages 1.2 kilograms (kg) pure seeds per hectoliter of closed cones.
6. There are 12,000 seeds per kilogram.
7. Laboratory germination is 80 percent; 50 percent of the germinated seeds produce plantable seedlings.
8. Cones are put into the kiln when they reach 25-percent moisture content.
9. Drying trays hold 0.5 hL of closed cones; each stack of eight drying trays holds 4.0 hL; eight stacks can fit in the kiln at once.
10. It takes 12 hours to dry a full charge to the 10-percent cone moisture needed for cones to open fully.

11. It takes 700 Kcal to heat 1 hL of cones for 1 hour.
12. Fuel value of wood of *Casuarina equisetifolia* is 4,950 Kcal per kilogram; for *P. roxburghii* cones, 4,500. Open *P. roxburghii* cones weigh 110 g per liter (L).

Questions:

1. How many cones must be collected to meet the planting goal?
2. How much total moisture must be lost in predrying (prior to entering kiln)?
3. How many drying stacks will be needed to predry everything at once?
4. How many kiln charges will be needed?
5. How long will it take to open all cones?
6. How much fuel will be needed with *C. equisetifolia* wood? With *P. roxburghii* cones?
7. Have enough cones been collected to heat the kiln?

Exercise 4 – Storage Space Requirements

Objectives:

Once annual seed requirements are known, space requirements for cold storage must be calculated. A decision must also be made as to how many years' supply of seeds will be maintained as a safety margin: 1 year's? 3 years'?

Assumptions:

1. You must grow 2 million *Acacia nilotica* and 3 million *Pinus wallichiana* seedlings each year.
2. A 3-year supply of seeds will be stored.
3. Number of seeds per kilogram (kg) is 7,000 for *A. nilotica* and 26,000 for *P. wallichiana*.
4. For every three seeds planted, only two will produce a plantable seedling.
5. The seeds will be stored in large plastic bottles that hold 10 kg each. The bottles are 80 centimeters (cm) tall and 40 cm in diameter.
6. Ten percent of the cold storage space is in aisles, etc.

Calculate:

1. How many kilograms of each species are to be stored?
2. How many bottles and cubic meters of storage space will be required?
3. Repeat calculation 2 if storage is in boxes 40 by 40 by 40 cm. Each box will hold 6.5 kg of seeds.
4. What are the minimum cold storage dimensions needed to store the seeds in calculations 2 and 3 above?

Exercise 5 – Sampling

Objective:

To learn the basic methods of sampling bulk lots and some special applications for tree seeds.

Methods:

1. Mix each lot thoroughly either with a mechanical mixer or by hand. To do the latter, spread the seeds out on a smooth surface and mix by scooping from side to side. Then pour back and forth between two containers.
2. Determine the proper size of the submitted sample (twice the working sample).

3. Draw samples using the following equipment/methods:
   a. Seed trier
   b. Mechanical divider
   c. Division
   d. Extended hand
4. Weigh each sample to the nearest gram, place in a plastic bag, and label.
5. Save these bagged samples for later measurements of purity, weight, and moisture.

Supplies:

A seed trier, a mechanical divider, a spatula, a spoon, plastic bags (15 by 15 cm), marking pens, and laboratory balances.

Exercise 6—Moisture, Purity, and Weight

Objective:

To carry out the basic steps in measuring moisture, purity, and weight of a submitted sample.

Methods:

1. Moisture
   a. Use submitted samples drawn in the sampling exercise.
   b. Use a spoon or spatula to draw two subsamples of 4 to 5 g each. Place samples in drying cans. Follow guidelines in Bonner (1981b).
   c. Weigh to the nearest 0.01 g and dry in ovens for 17 hours at 103 °C.
   d. Cool in desiccators and reweigh. If desiccators are not available, use rapid-weigh techniques to obtain dry weight.
   e. Calculate moisture as a percentage of wet weight:
   $$\text{percent moisture} = \frac{\text{wet wt.} - \text{dry wt.}}{\text{wet wt.}} (100)$$
2. Purity
   a. Reduce the remainder of the submitted sample to the proper working sample size. To determine the proper size, take at least 2,500 seeds up to a maximum of 1,000 g.
   b. Weigh the working sample (see 3.5.1.A in ISTA 1985).
   c. Divide the sample into the following components:
      (1) Pure seeds
      (2) Other seeds (other species)
      (3) Inert matter (includes seed parts)
   d. Weigh each component and express as a percentage of the working sample weight:
   $$\text{percent pure seed} = \frac{\text{wt. of pure seed}}{\text{wt. of entire sample}} (100)$$
3. Weight
   a. Use the pure seed component from the purity test.
   b. Either weigh and count the entire pure seed component or use smaller replicates (the usual method).
   c. Replicate method:
      (1) Randomly count out 8 replicates of 100 seeds each.
      (2) Weigh each replicate to the same number of decimal places used in the purity determination.
      (3) Obtain the mean weight of 100 seeds and multiply by 10 for 1,000-seed weight.

(4) Convert to pure seeds per kilogram as follows:

$$\frac{1{,}000{,}000}{\text{wt. of 1,000 seeds}} = \text{seeds per kg}$$

d. In official testing, variation would be estimated as follows:

(1) Variance $= \dfrac{n(\sum x^2) - (\sum x)^2}{n(n-1)}$

(2) Standard deviation $(\sigma) = \sqrt{\text{variance}}$

(3) Coefficient of variation (CV) $= \left(\dfrac{\sigma}{\bar{x}}\right)(100)$
($\bar{x}$ = mean wt. of 100 seeds, see 3.c above)

(4) If CV is 4.0 or less, the answer in "3.c" above is acceptable. If CV is more than 4.0, take 8 more replicates and repeat the process, using all 16 replicates in the calculations.

Supplies:

A spatula, a spoon, laboratory balances, an oven, drying cans, desiccators, forceps, and pencil and paper.

Exercise 7 – Calibration of Electric Moisture Meters

Objective:

To demonstrate a simple method for developing calibration charts for electric moisture meters. These methods will work with any type of meter.

Methods:

1. Draw 4 to 5 kg of seeds from a bulk lot of the desired species; mix well.
2. Separate into 10 random samples of about 400 g each.
3. Adjust moisture in these samples to span the range of moisture content that will be encountered (approximately 5 to 20 percent). Do this by drying several samples (vary drying conditions) and by adding water to others (vary the amount of water).
4. Place each sample in a plastic bag and place the bag in a cooler for 1 week to allow complete moisture equilibration.
5. After 1 week, remove the samples and let them come to room temperature (2 to 3 hours).
6. Take a meter reading on the driest lot according to the manufacturer's instructions. Record the value and immediately draw two 5-g subsamples for oven determinations of moisture content. Follow previous instructions.
7. Repeat step 6 with the other samples in the order of ascending moisture content.
8. Plot data on a graph: oven moisture percentage vs. meter reading. Use this curve to relate future meter readings to actual moisture content for this particular species only.
9. For more accurate calibration, fit a regression curve (oven moisture percentage on meter readings) and calculate values for a calibration table. More than 10 observations should be available for a regression, so another 10 samples should be drawn for a repeat of the entire process.
10. This procedure must be done separately for each species to be tested.

Supplies:

An electric meter, a spoon, laboratory balances, an oven, weighing dishes, desiccators, plastic bags, and graph paper.

Exercise 8 – Germination Tests

Objective:

To learn the basic steps of a germination test and to carry out simple tests on some important species. Because of the length of this course, a full test may not be possible. By starting a few samples before talking about testing at length, some germination should occur and be available for evaluation.

Methods:

1. Presoak seed samples in tapwater at room temperature (27 °C) for 15 to 24 hours.
2. Divide samples into 2 replicates of 20 to 50 seeds each, depending on the species. If *Eucalyptus* spp. seeds are used, weigh out two replicates according to ISTA (1985) rules.
3. Half the class will surface-sterilize their samples with a 10-percent chlorine bleach solution, and the other half will not treat theirs. Treatment will consist of a 5- to 10-minute soak, followed by rinsing in running tapwater.
4. Hard-seeded species (e.g., *Acacia)* will be scarified with a knife, file, or sandpaper on the radicle end as determined in Exercise 1.
5. Place replicates in glass or plastic dishes on moist filter paper or other suitable media. Paper should be moist, but not moist enough to leave "free water" in a depression made by mashing down on the paper with a finger. Put dish covers on; if there are no covers, use plastic wrap.
6. Label all dishes and place them in a germinator or constant temperature room if available. If these facilities are not available, place the dishes on a table under lights in the center of the room. If good lights are not available, place the dishes near windows that allow good natural light.
7. Check dishes every day for moisture; add water if they dry out. Germination may become evident in about 7 days. Record normal germination, abnormal germination, and evidence of insect or disease problems.

Supplies:

A knife, small file, or sandpaper for scarification, chlorine bleach, germination blotters, dishes (10 per student), a glass-marking pen, and laboratory balances. A germinator or constant temperature room is desirable but not necessary.

Suggested species:

Pinus, Acacia or another legume, *Eucalyptus,* and two indigenous species.

Exercise 9 – Scarification

Objective:

To demonstrate the relative effectiveness of simple scarification techniques that can be used in seed testing.

Methods:

1. Count out 120 seeds of a hard-seeded species and divide them into 8 samples of 15 seeds each.
2. Scarify 2 replicates of 15 by each of the following procedures:
   a. Rub hand files or similar abrasive devices across the seedcoat enough to cut a notch in the seed.
   b. Use hand clippers, shears, or a knife to cut through the seedcoat along one side.
   c. Sandpaper the seed enough to cut through the seedcoat on the radicle end.
   d. The other two samples will be the untreated controls.

3. Place the scarified samples on moist blotters in dishes and cover as in the germination test. Place all dishes in the germinator if there is space. If not, place them on a table with good lighting and leave them for observation through the rest of the course.
4. Periodically count the number of germinating seeds and the number of swollen seeds. This latter condition confirms that water uptake has occurred, but something else may be blocking germination. Report both conditions as a percentage of the total number of seeds in the test.

Supplies:

Four glass or plastic dishes, germination blotters, a hand file, clippers or shears, rough sandpaper, and marking pens.

Exercise 10 – Rapid Test: Tetrazolium Staining

Objective:

To learn basic techniques of the tetrazolium (TZ) stain test for viability.

Methods:

1. Draw two 50-seed samples from seeds that have been soaking in tapwater for 24 hours.
2. Prepare a 1-percent solution of TZ by dissolving 10 g of a TZ salt (chloride or bromide) in 1,000 mL of distilled water (pH 6.5 to 7.0). If the pH of the water is outside this range, a buffered solution must be prepared as follows:
   a. Prepare two solutions:
      (1) Solution 1 – Dissolve 9.078 g KH_2PO_4 in 1,000 mL of water.
      (2) Solution 2 – Dissolve 11.876 g $Na_2HPO_4 \cdot 2H_20$ in 1,000 mL of water.
   b. Mix two parts of solution 1 with three parts of solution 2.
   c. Dissolve 10 g of TZ salt in 1,000 mL of the buffer solution to make a 1-percent solution.
3. Carefully cut open the imbibed seeds to fully expose the embryo. The embryo may be completely removed, as in the excised embryo test.
4. Completely immerse the embryos in TZ solution in dishes and incubate them in the dark at 30 °C for 15 to 24 hours (depending on the species and seed condition).
5. For evaluation, decant the TZ solution, rinse seeds in water, and examine the embryos on a wet surface. Moderate red staining generally indicates viable tissues, heavy red staining indicates damaged tissues, and the absence of any staining indicates nonviable tissues. Stain interpretations may vary by species. See ISTA (1985) for guidelines.
6. Compare results with other rapid test results or germination test results.

Supplies:

Dissecting equipment, dishes, tetrazolium salt, buffers (if necessary), and a constant-temperature dark incubator.

Exercise 11 – Rapid Tests: Cutting and Excised Embryo

Objective:

To learn techniques of the cutting test for viability estimation and of embryo removal for the embryo excision test.

Methods:

1. Cutting Test
   a. Draw samples of 50 seeds from each of several seedlots and divide them into sublots of 25 seeds each.
   b. Cut the seeds in half, using a transverse cut through the center of the seeds. Categorize seeds as either viable, damaged by insects or disease, or empty. Average the results from the sublots.
2. Excised Embryo Test
   a. Draw samples of 50 seeds from each of several seedlots that have been soaking in tapwater for 24 to 48 hours at room temperature and divide into sublots of 25 as before.
   b. Using razor blades or scalpels, carefully cut through each seedcoat and endosperm (if present), and expose the embryo.
   c. Carefully "tease" the embryo out of the surrounding tissues with dissecting needles or other sharp-pointed instruments. Avoid damaging the embryo.
   d. Carefully place the excised embryo on moist filter paper in a covered dish, such as a petri dish. Maintain at 20 °C in light until an evaluation can be made (usually within 14 days).
   e. Diseased or damaged embryos should not be placed in the dishes. Empty seeds should be categorized as such and not replaced in the test.
   f. The working surface and all instruments should be disinfected to reduce mold infections with a 50-percent ethanol solution. Instruments should be "dipped" between each dissection.
   g. Embryos should be categorized within 14 days as follows:
      (1) Viable
         (a) germinating embryos
         (b) embryos with one or more cotyledons exhibiting growth or greening
         (c) embryos remaining firm, slightly enlarged, and either white or yellow according to species
      (2) Nonviable
         (a) embryos that rapidly develop severe mold, deteriorate, and decay
         (b) degenerated embryos
         (c) embryos exhibiting extreme brown or black discoloration, an off-gray color, or white watery appearance
         (d) seeds in which the embryo is dead, missing, or deformed
   h. Compare your results with the cutting test results.

Supplies:

Single-edged razor blades, scalpels, dissecting needles, dishes, filter paper, and ethanol.

Exercise 12 – Seed Health Testing

Objective:

To learn basic techniques of seed health testing.

Background:

Health testing of seeds is important for three reasons:
1. Seed-borne inoculum may cause diseases in the field.
2. Imported seedlots may introduce new diseases, so tests to meet quarantine regulations may be required.
3. Seed health testing may aid in seedling evaluation and help determine causes for poor germination or field establishment. It supplements the germination test.

Seed Health refers primarily to the presence or absence of disease-causing organisms (e.g., fungi, bacteria, and viruses) and animal pests (e.g., eelworms and insects). However, physiological conditions such as trace element deficiency may be involved.

Incubation maintains seeds in an environment favorable to the development of pathogens or symptoms.

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Pretreatment is any physical or chemical laboratory treatment of the working sample preceding incubation that is done solely to facilitate testing.
Treatment is any process, physical or chemical, to which a seedlot is submitted.

Sample:

1. Entire submitted sample may be the working sample, depending on the test.
2. The working sample is normally 400 pure seeds or an equivalent weight.
3. Sampling rules are followed.
4. Replicates containing a specified number of seeds, if required, are taken at random for a subsample after thorough mixing.

General directions:

1. Use different methods of testing depending on factors such as pathogen or condition being investigated, species of seeds, and purpose of test. See ISTA (1966, 1985).
2. Examine the working sample with or without incubation.
   a. Examine without incubation. (This method provides no indication of the viability of the pathogen.)
      (1) Examine the sample with a stereomicroscope for general evidence of diseases or pests.
      (2) Examine imbibed seeds. Immerse the working sample to make fruiting bodies, symptoms, or pests more easily visible and to encourage the release of spores. Examine with stereomicroscope after imbibition.
      (3) Examine organisms removed by washing. Immerse the working sample in water with a wetting agent, or in alcohol, and shake to remove spores, hyphae, nematodes, etc. Examine the excess liquid with a compound microscope.
   b. Examine after incubation.
      (1) After a specific period of incubation, examine the working sample. Note the presence of disease organisms or pests on or in seeds or seedlings. Use blotters, sand, or agar for incubation media.
      (2) Use blotters when required to grow the pathogens from the seeds or to examine the seedlings. Seeds may or may not be pretreated. Space widely to avoid secondary spread of organisms. Use light as necessary to stimulate sporulation. Examine with a microscope.
      (3) Sand or artificial composts can be used for certain pathogens. Seeds are not usually pretreated, but they are widely spaced on the medium. Incubation is favorable for symptom expression.
      (4) Use agar plates to obtain identifiable growth of organisms from seeds.
         (a) Sterility is required; seeds are normally pretreated and spaced.
         (b) Identify characteristic colonies and spores by microscopy.
         (c) Use lighting and germination inhibitors.
3. Examine growing plants. Grow plants from seeds and examine them for disease symptoms to determine the presence of bacteria, fungi, or viruses. Use inoculum from the test seedlot to test for infection of healthy seedlings.

Calculations and Expression of Results:

1. Express results as a percentage of seeds affected or as number of organisms in the weight of sample examined.
2. Report results on the ISTA certificate.
   a. Report test method.
   b. Report pretreatments.
   c. Absence of health test does not imply satisfactory health condition.

Specific Test Example – Pitch Canker Fungus:

1. Adapted from Anderson (1986b).
2. Blotter Method, 400-seed sample.

a. Pentachloronitrobenzene (PCNB) Broth
Combine peptone, 15 g; $MgSO_4$ $7H_2O$, 5 g; KH_2PO_4, 1 g; terraclor, 1 g with 1 L of distilled H_2O. Stir well using magnetic stirrer. Autoclave for 15 minutes. After autoclaving, place flask on magnetic stirrer and stir slowly until solution cools to room temperature or slightly warmer. Add 1 g of streptomycin sulfate and 1 to 2 g of neomycin sulfate under sterile conditions, and stir.

b. Place 25 seeds on blue blotter paper in plastic containers. Crush the seeds with a sterilized piece of plastic cut to fit the plastic box opening. Spray seeds and blotter paper with PCNB broth.

c. Incubate 14 days at 20 °C or until colonies are 2 cm in diameter.

d. Inspect all seeds for slow-growing, granular white colonies. Check each suspected colony using a light microscope at 100 to 400 magnification for microconidia and polyphialids. Select fungus from seed surface, not the blotter surface. Split the seeds into 4 groups of 100 for reporting purposes.

3. Agar Method

a. Prepare fresh potato dextrose agar (PDA) (makes 1 L)

(1) Clean and dice one medium-sized potato.

(2) Put diced potato in beaker with 500 mL of distilled H_2O. Run through autoclave.

(3) In flask, add 20 g of dextrose and 17 g of agar to 500 mL of distilled H_2O.

(4) Put dextrose/agar solution on magnetic stirrer and low heat.

(5) Strain cooked potatoes through two layers of cheesecloth to obtain at least 200 mL of slurry.

(6) Note amount of slurry, and pour slurry in flask with dextrose/agar solution.

(7) Add enough distilled H_2O to make total slurry solution amount to 500 mL (i.e., if there are 200 mL of slurry, add 300 mL of distilled H_2O).

(8) Put solution in autoclave and run for 15 minutes. To acidify media, add 20 drops of 50 percent lactic acid to obtain a pH of 4.7.

b. Isolating external seed fungi, 25-seed sample

(1) Place the whole seeds on acidified PDA (pH 4.7).

(2) Incubate 14 days at 20 °C.

(3) If possible, observe fungal growth daily and identify the fungi.

c. Isolating internal seed fungi, 25-seed sample

(1) Surface sterilize the whole seeds in 70 percent ethanol for 10 minutes. Stir the seeds every 2 minutes.

(2) Under sterile conditions, cut each seed open and remove half the center material.

(3) Place the seed half (center material) on acidified PDA (pH 4.7) using sterile technique.

(4) Incubate 14 days at 20 °C.

(5) If possible, observe fungal growth daily and identify the fungi.

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